STUDIES ON INTERACTION OF ROOT-KNOT NEMATODES AND ROOT-INFECTING FUNGI ON AIR POLLUTION STRESSED LEGUMINOUS CROPS

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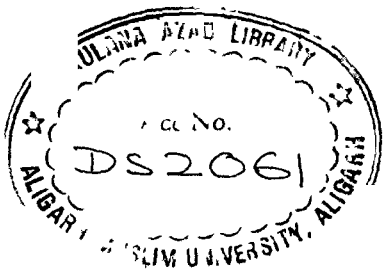

Dedicated

to

My Parents

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Certificate

This is to certify that **Mr. Syed Abbas Hosseini Negad** has worked in this centre as a Research Scholar under my guidance. His dissertation on **Studies on interaction of root-knot nematodes and root-infecting fungi on air pollution stressed leguminous crops** is original and upto-date. He is allowed to submit this dissertation for consideration of the award of the degree of **Master of Philosophy** in **Agriculture (Plant Pathology)**.

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INTRODUCTION

Pulses are one of the most important sources of protein in human dietary system. Their importance becomes more appreciable for vegetarian populations particularly in India, otherwise they would suffer from protein deficiency. Pulses are used as a food in a variety of forms. The most common is the soup, the split pulse grain being boiled in spiced water, grains (seeds) may be ground and boiled, roasted or fried.

Pulses are also grown as fodder crops. Their grains are used also as concentrates to enrich the diet of the animals. Some pulse crops grown also as green-manure crops and are excellent for this purpose. They fix atmospheric nitrogen symbiotically in association with root nodules bacteria and show no dependence on nitrogen fertilizers. Their ability to thrive under a wide range of soil and climatic conditions, specially with their deep root system and ability to withstand dry conditions more successfully than most other commonly grown crops are excellent. Therefore, some of the pulse crops are suitable for dry farming and can figure prominently in soil conservation programmes.

One of the most important pulse crop grown in India is gram (*Cicer arietinum* L.). It is a multipurpose pulse crop grown throughout the country for various uses. Its leaves are

as a vegetable, spiced and cooked. In general, it is a rich source of protein, fat, carbohydrate and vitamins.

Soybean, *Glycine max* (L.) Merr., is another important legume grown mainly as a food crop in various parts of the world. In recent years, soybean has attained great importance in the U.S.A which shows the highest acreage under this crop. The cultivation of this crop in India is also getting significance. Because of its high nutritional values and good quality of oil, soybean seeds have been put to a wide range of uses through-out the world. Seeds of soybean are consumed green, dry or sprouted, besides making various products such as soymilk, soysauce, cheese called 'Tofu' and curd. The soybean milk can be used for feeding infants. In the U.S.A. although it is grown essentially as an oil seed crop and more than 90 percent of the produce is annually crushed for oil, it has vast diversification of industrial ulitization. The commercially important by-products of oil industry include lecithin, soap stock and oil meal for livestock and poultry; and the edible ones comprise margarines, shortening and salad oils. The non-edible outlets are paints, varnishes, inks, oil cloth, enamel and linoleum.

Recently attention of the Indian Council of Agricultural Research was drawn to the problem of pulses in India and an integrated All India Coordinated Programme for

the improvement of pulse crops has been undertaken. The work is being done in collaboration with U.S. Department of Agriculture at different centres in India.

The nutritive quality of the pulse grain is an important aspect of research work which is considered by the plant breeders from the beginning of the programme. The plant breeders has also considering the disease and pests problems which causes severe damage to the crops.

Like many other crops pulses are attacked by a number of plant pathogen. Fungi, bacteria, viruses and nematodes are common pathogens that cause an array of diseases of economic consequences. The most important fungi attacking the aerial parts of the crops are *Ascohyta rabiei* causing blight, *Uromyces phaseoli* (rust), *Alternaria* spp. (leaf blotch), *Botrytis* spp. (chocolate spot), *Erysiphe polygoni* (powedy mildew), *Stemphylium* spp. (Chlorosis of leaves), *Cercospora* spp., *Pleospora herbarum*, *Colletotrichum limdemuthianum* f. sp. *phaseoli* (anthracnose). *Colletotrichum destructivum*, *Leviuellula taurica* Lev. (powdery mildew), *Oidium* spp. and *Helminthosporium* spp. The fungal pathogen of roots of pulses are *Rhizoctonia bataticola*, *R. solani*, *Fusarium oxysporum*, *F. lateritium*, *F. culmorum*, *F. avenaceum*, *Phythium* spp., *Phialophora* spp. *Peyronelleae* spp. and *Sclerotium rolfsii*. Among the bacterial diseases of pulses, bacterial blight caused by *Pseudomonas glycinea* Coerper, bacterial pustule

caused by *Xanthomonas phaseoli* and wildfire caused by *Pseudomonas tabaci* (Wolf and Foster) F.L. Steven, are the most important ones. Pulses also suffer from viral diseases too. Some examples are Bean Common Mosaic Virus (BMV), Common Aphid transferred Mosaic virus (CAMY), Bean Yellow Mosaic Virus (BYMV), Bean Mosaic Virus (BMV), and Pea Mosaic Virus (PMV).

Among the fungal disease of chick-pea and soybean, *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Chattopadhyay and Sen Gupta and *Rhizoctonia bataticola* (Taub.) Butler (=*Sclerotium bataticola* Taub.) causing wilt and root-rot of their respective hosts have been found to be the most serious in India and are often responsible for "soil sickness" problem when the soil becomes heavily contaminated with them due to continuous crop cultivation.

A variety of plant parasitic nematodes have been found in association with pulses. Species of *Heterodera*, *Meloidogyne*, *Rotylenchulus*, *Tylenchorhynchus*, *Aphelenchoides*, *Xiphinema*, *Helicotylenchulus*, *Hemicriconemoides*. *Paraphelenchus*, *Paratrichodorus*, *Hoplolamis*, *Hirschmaniella*, *Macroposthonia*, *Pratylenchus*, *Criconemoides*, *Ditylenchus*, *Diptherophora*, *Tylenchus*, *Caloosia*, *Telotylenchus*, *Aglenchus*, and *Orisitylus* are recorded on pulses (Singh and Gill, 1990).

Among all mentioned above, root-knot nematodes, *Meloidogyne* spp. with a world-wide distribution and diversified host range are a serious pest of pulses. They attack almost all the pulses and their pathogenicity have been established. *Meloidogyne incognita* causes a significant reduction in growth parameters of mothbean, *Vigna aconitifolius* Jacq. (Mishra and Gaur, 1981a), french bean, *Phaseolus vulgaris* (Singh *et al.*, 1981), black gram, *Vigna mungo* (Mishra and Guar, 1981 b), cluster bean, *Cyamopsis tetragonaloba* (Verma *et al.*, 1981), pea, *Pisum sativum* (Upadhyay and Kusum Dwivedi 1987), cowpea, *Vigna unguiculata* (Varshney *et al.*, 1981), winged bean, *Psophocarpus tetragonabola* (Nayak *et al.*, 1987), soybean, *Glycine max* (Nalini *et al.*, 1985; Kinloch, 1980), chick-pea, *Cicer arietinum* (Mani and Sethi, 1987; Kumar *et al.*, 1988). *M. javanica* also attacks pulses e.g. chick-pea (Goel and Gupta 1984, 1986), pigeonpea, *Cajanus cajan* (Ngauma and Saka, 1986; Abdul Salam and Khan 1986), blackeye bean, *Vigna sinensis* (Thomason, 1958). *M. arenaria* also is a limiting factor in production of soybean (Kinloch *et al.*, 1987; Rodriguez-Kabana and Williams, 1981). Soybean cyst nematode. *Heterodera glycines* has been reported to be one of the most widespread and damaging nematodes of soybean (Kinloch, 1980; Wrather and Anand, 1988).

Females of root-knot nematodes are sedentary endoparasites. Cells around the head and neck of the female nematodes becomes multinucleated and cell wall irregularly thickened. Usually 6-12 of such cells are found around the neck of each female which function as transfer cells (Jones and Northcote, 1972b) and female nematodes obtain their nutrition from them. Vascular tissues of the plant are altered extensively due to invasion of roots by root-knot nematodes which impair the absorption and translocation of water and nutrients to vegetative parts of the plant. Suppression of plant growth and appearance of deficiency symptoms on leaves are due to biochemical changes induced by the nematodes in the host and altered physiology due to the anatomical transformations.

The second stage juvenile (J_2) of the nematode penetrates into the root and moves through the cortex inter- and intracellularly till it reaches close to the pericycle. The female become saccate and sedentary and mainly responsible for pathogenic damages caused to the host plants. Root-knot nematode interacts with soil-borne plant pathogens. Synergistic interactions of root-knot nematodes and several soil-borne fungi are well documented Powell, 1971, 1979; Khan, 1984; Taylor, (1979, 1991). Root-knot nematodes suppress the formation of root-nodulation in pulses (Dhanger and Gupta, 1983: Rauti, 1980; Mani & Sethi 1984,

1987), as a result of which the total benefit from the nitrogen fixing bacteria is reduced.

Atmospheric pollution is a major problem of the modern world. The fast increasing human population and quest for improvement in living standard have resulted into rapid industrialisation, urbanisation, excessive use of fertilizers and pesticides on major agricultural crops and production of nuclear energy. Consequently, every component of the biosphere i.e. the air and the soil, is becoming tremendously polluted by day and night. According to the estimates of Steve Van Matre (1984), in the present world only 20% of the air is breathable and only 10% of the land is capable of being exploited for producing food. Astanin and Blagosklonov (1983) reported that hundreds of millions tons of harmful gases and dusts are emitted in to the earth's atmosphere every year.

The release of harmful chemicals and toxic materials from industries into the environment (air, water, soil) cause damaging effects on both fauna and flora. These toxic wastes in excess, exceeding the normal self-regulating capacity of atmosphere, lead to pollution. Weber (1982) defined air pollution as 'the presence of substances in the ambient atmosphere, resulting from the activity of man or from natural processes, causing adverse effects to man and the

environment". In 1966, the committee on pollution, National Academy of Sciences, U.S.A. defined the pollution as an undesirable change in the physical, chemical or biological characteristics of our air, land and water, that may or will harmfully affect human life or that of desirable species, our industrial processes, living conditions and cultural assets, or that may or will waste our raw material resources (Anonymous, 1966). In other word, any undesirable change in the physico-chemical and biological properties of air, water and soil, which may cause harm to man, other organisms, or the cultural and natural elements of man's environment, is pollution.

Environmental pollution includes many kinds of pollutions such as soil pollution, water pollution, noise pollution and air pollution. However, the last one probably has attracted the attention of the world most. Wood (1968) classified the toxic substances responsible for air pollution "air pollutants" basically in two categories based on their origin. Primary air pollutants, those originating at the source in the form of toxic to living organisms which may be in gaseous or particulate forms. Gaseous air pollutants are sulphur dioxide (SO_2), oxides of nitrogen (NO_x), hydrogen fluoride (HF), ammonia (NH_3), ethylene ($C_2 H_6$) etc. and particulate air pollutants are coal dust, cement dust, fly ash, suspended particulate matter (SPM) etc. The second

category of pollutants are secondary air pollutants which originates from the reaction between primary air pollutant that originate from the source e.g. photochemical pollutants like peroxyacetyl nitrate (PAN) and ozone (O_3). SO_2 and NO_x in high humid conditions are converted into acid (H_2 SO_4 and HNO_3) which fall on the ground in form of 'acid rain' during the atmospheric precipitation (Oden, 1968).

Sulphur dioxide is one of the most damaging air pollutant for plants. Sulphur dioxide make entry through stomata to reach the mesophyll of the leaves and thus having come in contact with water, change in H_2 SO_3 or HCO_3^- if the medium is acidic, or SO_3^{-2} - SO_4^{-2} when alkaline, which damages to adjoining tissues making for chlorosis and drying of leaf tips, etc.

Plants are affected by the presence of gaseous pollutants in the air. The performance of the plants is influenced directly or indirectly by inhibition or acceleration of the plant metabolism, which may influence their productivity. Many significant and sometimes devastating effects of air pollution of vegetation have come to light during the current century. Some earlier reports have revealed the devastating effect of SO_2, fluoride, hydrogen chloride and other pollutant on plant population. Recently Temmerman (1982) described the effect of ammonia on vegetation, following an accidental release of the gas.

Astanin and Blagosklonov (1983) concluded that various afflications of plant leaves appear under the influence of air pollutants and the plant growth is stunted. Leaves are probably the most sensitive plant parts to air pollutions. They first develop certain protective adaptation and ultimately fall prey to the harsh atmosphere. Stomata and trichomes usually undergo changes pertaining to size and frequency. Photosynthesis, a process responsible for sustenance of the whole plant body is much vulnerable to air pollution damage. The influence of toxic gases on agricultural crops is little studied. However, in some industrialized countries air pollution is known to reduce the yield of many crops near industrial areas. Plants affected by sulphur dioxide are poor in protein; the sugar beats and potatoes show reduction in sugar and starch respectively. The greatest harm to U.S. agriculture is inflicted by phot-oxidants. For example, in the Los Angeles, Valley farmers were forced to cease growing certain vegetables (spinach, lettuce) most susceptible to photo-oxidants (ozone and peroxyacetyle nitrate), and the citrus fruit yield decreased by 50% (Astanin and Blagosklonov, 1983).

Deposition of particulate air pollutant on leaf surface blocks the stomatal cavities which hampers transpiration and checks the transmission of solar radiation (Darley, 1966).

Their deposition reduces plant vigour, growth and productivity.

In agricultural field plots, plants are exposed to a variety of biotic and abiotic pathogens, especially roots, which are constantly exposed to many soil microorganisms. According to Richards (1976), one square meter of highly fertile field soil may contain as many as 3×10^{14} bacterial cells (300g), 5×10^{8} protozoa (39g), 1×10^{7} nematodes (12g), and 400g of fungi. However, this can not generalized for all soils, and many microbes present are saprophytic or have weak pathogenic potential to plants. Many investigators have concluded that the majority of root diseases have a complex etiology (Grogan 1981; Wallace 1978). Fawcett (1931) stated that nature does not work with pure cultures and that most plant diseases, particularly root diseases, are influenced by associated micro-organisms. Root infections by one pathogen may modify the host response to subsequent infections by pathogen or saprophytes.

Nematodes and soil-borne micro-organisms thrives best under moist soil condition. A number of excellent reviews on the interactions of plant-parasitic nematodes with other plant pathogens have been written by Bergeson, (1972) Pitcher (1963, 1965, 1978), Powell (1963a, 1963b, 1971a, 1971b, 1979), Taylor, (1979, 1991) and Wallace, (1978). The

reviews analyse many specific interactions and in most interactions involving fungi and nematodes, nematode was not essential for the establishment and development of the fungal pathogen. The nematode usually assisted the fungus by altering the incidence and speed of development of the pathogen and thus increasing the disease severity. Thus, the interaction between the nematode and the fungal pathogen is often indirect and occurs owing to induced modifications in the host plant. In case of ectoparasitic nematodes, host-plant modification may be minimal, whereas in sedentary endoparasitic nematodes such as root-knot nematodes, these modifications are extensive and complex. Bowman *et al.*, (1966) reported both localized and systemic host-tissue modifications through the production of translocable metabolites by nematodes. However, the nematodes and the fungal pathogens may occasionally act directly on each other.

Another type of multiple-pathogen interaction is the interaction between biotic and abiotic (air pollutants) pathogens. Air pollutants are emitted to the atmosphere from the point sources continuously and spread in all directions depending upon direction and velocity of the wind, affecting plants growing in the vicinity. Therefore on air pollutions stressed plants, associated microorganisms including plant parasites are likely to be affected. At present, very little is known of the effect of pollutants on parasitic diseases

of plants and host parasite relationships. Some studies however, have been undertaken to determine this aspect of air pollution impact. Theoretically, parasitism may be affected by air pollutants in different ways. Parasitism may be increased or decreased through a direct effect of pollutant on the parasite, or the effects may be indirect through pollutant - induced changes in the host plant. Sulphur dioxide may act directly upon the fungi or indirectly through some effect upon living or dead plant tissue, soil or water. Studies undertaken show that fungi differ in their reaction to SO_2; rust diseases and wood-destroying fungi are sensitive to SO_2. Wheat stem rust, *Puccinia graminis* was less in industrialized area than unindustrialized areas (Johanson, 1954). Scheffer and Hedgcock (1955) found decreased parasitism by species of *Cronartium*, *Coleosporium*, *Melampsora*, *Peridermium*, *Pucciniastrum*, and *Puccinia* where trees were injured by SO_2. Sulphur dioxide affects fungal growth too. Hyphae of different species vary in sensitivity to SO_2 exposures (MacCallan *et al.*, 1940).

Pollutants also affect the nematode parasitism. Reproduction of *Heterodera glycines* and *Paratrichodorus minor* was decreased by O_3 alone or in mixture of SO_2, but that of *Belonolaimus longicaudatus* and *Pratylenchus penetrans* will not altered (Weber *et al.*, 1979). Reproduction of *P. penetrans* on tomato was slightly suppressed by O_3 at 0.20 ppm

but not by SO_2 at 0.80 ppm (Brewer 1979). Kozlowska (1981) observed that industrial dust has harmful effect on *Panagrolaimus rigidus*. Bisessar *et al.*, (1984) found that root-knot nematode (*M. hapla*) infected tobacco plants were more sensitive to ambient ozone. However, non-injurious doses of SO_2 failed to affect adversely the plant-parasitic nematodes. Reproduction of *Pratylenchus penetrans* was increased when plants were exposed to SO_2 before nematode inoculation. This dose of SO_2 was not enough to injure leaves or significantly to affect plant growth. Singh (1989) observed that incidence of infection of chick-pea by *M. incognita* and *M. javanica* were less around the pollution source. The incidence of disease was decreased with the increasing distance from the pollution source. He observed that air pollution in general inhibited the root-knot disease on pulse crops in ambient condition. The extent of inhibition was correlated with the ambient levels of pollutants. He noted that juvenile hatching of both species were adversely affected by SO_2, O_3 and their mixture. Higher concentrations of each pollutants individually or in mixture were more inhibitory to juvenile hatching and *M. incognita* was more sensitive than *M. javanica* to pollutants. However, much is not known about such interaction and needs more investigation. Although some studies have been undertaken to determine the effect of air pollution on nematodes or fungal disease, the impact of air pollution stress on development of

fungus-nematode complexes on crop plants is yet to be investigated.

The main objective of the proposed work is to acertain the impact of air pollution caused by SO_2 on root-knot-fusarium wilt interaction on chick-pea and root-knot-root rot interaction on soybean.

Experiments will be carried out under artificial treatment conditions i.e. using exposure chamber for SO_2 treatments. The following aspects are proposed to be studied to achieve main objective of the study.

1. Pathogenicity test of root-knot nematode, *Meloidogyne incognita* on chick-pea and soybean cultivars.

2. Pathogenicity test of wilt fungus (*Fusarium oxysporum* f. sp. *ciceris*) on chick-pea cultivars and root-rot fungus, *Rhizoctonia bataticola* on soybean cultivars.

3. Effect of sulphur dioxide on chick-pea and soybean cultivars.

4. Effect of *M. incognita* infection to wilt of chick-pea caused by *F. oxysporum* f sp. *cicers* and root-rot of soybean caused by *R. bataticola.*

5. Interaction of *M. incognita* and *F. oxysporum* f. sp. *ciceris* on chick-pea and *M. incognita* and *R. bataticola* on soybean.
6. Interaction of root-knot nematode, *M. incognita* and SO_2 on chick-pea and soybean.
7. Interaction of SO_2 with *F. oxysporum* of sp. *ciceris* on chick-pea and *R. bataticola* on soybean.
8. Impact of SO_2 on interaction of *M. incognit* with *F. oxysporum* f. sp. *ciceris* and *R. bataticola* on their respective hosts i.e. chick-pea and soybean.

These aspects can be suitably modified depending upon the progress of the work and results obtained.

LITERATURE REVIEW

Total land under the cultivation of pulse crops in India is about 24 million hectare. with approximate production of 12 million tons annually. According to Vavilov (1951) India and Middle East form the primary centre of origin of most of the important legumes.

Chick-pea (*Cicer arietinum* L.) is the most important pulse crop grown in India which occupies an area of about 23 million acres, which is about three-fourths the wheat acreage of India. The chick-pea is perhaps indigenous to the South-east Europe. It is supposed that it was cultivated in Egypt from the early earliest time of Christian era where it was introduced from Greece and Italy. Its introduction into India is of a more early date. There is a Sanskrit name and several other names in modern Indian languages. It is considered to have originated in the tract lying between Caucasus and the Himalayas where from it has spread to the southern Europe, Persia (Iran), Egypt and India. It has also been introduced into parts of the central and southern America, Australia and parts of Africa. *Cicer* includes 22 species (Index Kewensis, 1913) distributed in the Mediterranean and West and Central Asia. In Asia minor and Egypt, they occur only in the wild state. *Cicer soongaricum* is cultivated in parts of the Western Himalayas. Grains of the two wild species *C.*

pinnatifidum Jaub & Spach (Avdulov, 1937) and *C. montbreii* Jaub & Spach are tiny; those of former have angular seeds with minute spines on the testa; while the grains of the latter have a smooth testa (Iynegar, 1935).

Soybean (*Glycine max* (L.) Merr. in believed to have originated from south-east Asia, derived from a twiny slender plant *G. ussuriensis* Regal and Macck, commonly growing in this region. According to Fukuda (1933), Manchuria should be the centre of origin since it shows diversity in forms in this country. Engelbert Kaemfer, a German botanist who was in Japan during 1690-92, was the first to introduce soybean to European World. The first mention of soybean in American literature is by Mease in 1804. Generally three species of *Glycine* are recognized, viz., *G. ussuriensis* (wild soybean), *G. max* (the cultivated form), and *G. gracilis* (intermediate type between the first two). It is believed that *G. ussuriensis*, formerly known as *G. soja*, is the progenitor of the cultivated soybean (Piper and Morse, 1910). It is procumbent type with twining stems and narrow leaf lets. Seeds are sooty dark in colour and range from 1-2g. per 100 seeds, while in the cultivated soybean the plant is erect or semi-erect and the seed colour varies from yellow, green, brown to black. The seed weight ranges from 5-35 g. per 100 seeds.

Air Pollution and Plants

All living organisms including plants are dependent on their environment. The environment constantly influences and is influenced by its inorganic and organic surroundings. The various gases and inorganic substances obtained from air, water and soil one converted into complex organic molecules by leaves of the plant. Presence of gaseous pollutants in the air affect the performance of plant directly or indirectly by inhibiting or accelerating the plant metabolism which may influence its productivity.

A considerable amount of gaseous air pollutants originating from different activities of man are released into air which impures atmosphere. Sulphur dioxide (SO_2), ozone -(O_3), oxides of nitrogen (NO_x) are recognised as the most important toxic gaseous air pollutants and harmful to the plants. Cameron (1874) observed that factory smoke which caused disease in plants contained SO_2. According to Heck *et al.* (1982) crop losses due to air pollutants may reach upto 90%. They suggested that it conducting agricultural research, air quality-agricultural plant ecosystem interrelationship should be taken into the consideration. Heagle (1973) reported that air pollutants alter the susceptibility of plants to biotic pathogens like fungi, nematodes, bacteria, etc.

It is now realised that the pollution damage is obvious, severe and inescapable unless the violation of the environment safety is not checked. Astanin and Blagosklonov (1983) have given a chart for maximum permissible concentration of harmful substances in the air over polluted area (Table I).

Effects of airborne sulphur pollutants on plants

Airborne sulfur pollutants which affect plant life are sulphur dioxide (SO_2); hydrogen sulfide (H_2S), and sulphuric acid ($H_2 SO_4$), and acid precipitation. The information is mostly available on SO_2, and the effects of this gas on forest trees, horticultural plants, and lichens have been documented. Effects of various sulphur pollutants have been reviewed by Thomas (1952), Daines (1968), Barrett and Benedict (1970), the US Environmental Protection Agency (1973), Heck and Brandt (1975), Varshney and Garg (1979), Amundson (1983), Bender *et al* (1986), Fluckinger and Younger (1986), Benwart (1987), Bytnerowicz *et al.*, (1987) and Kumar and Yadav (1988).

Sulphur is an essential element for plants. It is a constituent of the amino acids cysteine, and methionine. It is also a constituent of the plant vitamins, thiamine and biotin, and of other biochemical constituents such as glutathione, coenzyme A, and cytochrome C. Normally, sulphur

TABLE 1

Maximal permissible concentrations of harmful substances in the air over polluted areas.

Pollutant	Maximum permissible concentrations (mg/m^3)	
	Short term	Daily
Ammonia	0.20	0.20
Arsenic	-	0.003
Benzine	5.00	1.50
Benzol	1.50	0.80
Carbon disulphide	0.03	[illegible]
Carbon monoxide	3.0	1.00
Carbophos	0.015	-
Fluorine	0.10	0.03
Fluorine compounds	0.03	0.01
Formaldehyde	0.035	0.012
Hydrogen sulphide	0.008	0.008
Lead	-	0.0007
Mercury	-	0.0003
Nitrogen dioxide	0.085	0.085
Non-toxic dudt	0.50	0.15
Phenol	0.01	0.01
Phosphoric anhydride	0.15	0.05
Soot	0.15	0.05
Sulphur dioxide	0.50	0.05
Sulphuric acid	0.30	0.10

Short term = Emission mostly for 20-30 minutes.
Daily = Average daily emission over a long period.

is taken up by plants from soil in the sulphate form and assimilated into various compounds usually after being chemically reduced. Sulfur dioxide absorbed from the air has been shown to rapidly undergo oxidation to sulphates inside plant tissue. De Cormis (1969) found that tomato plants soon after exposure to SO_2 had 98% of the S in the form of sulphates.

Sulphur dioxide is emitted from various industrial sources and the adverse effects on vegetation in the vicinity of these sources have been well documented. Some of the industerial sources are -

1. Zinc and lead Smelters
2. Nickel and Copper Smelters
3. Iron concentrators
4. Petroleum refineries
5. Pulp and paper mills
6. Fossil fuel thermal generating stations

Estimation of the cost of air pollution damage to crops have varied considerably. Annual loss of $ 500 million have been estimated in the United States from air pollution injury to crop plants with approximately one third of this total loss attributable to SO_2 injury.

The first visible evidence of SO_2 injury to plants is discernible in the foliage. The stem, bud, and reproductive parts of the plants are visibly more resistant to SO_2. The effects of airborne sulphur pollutants on plants may be divided into three categories: acute, chronic, and subtle (physiological and biochemical). Sulphur dioxide enters leaves mainly through the stomata and is toxic to the metabolic processes taking place in the mesophyll cells. Acute injury is caused by a rapid accumulation of bisulphite and sulphite. When the oxidation product, sulphate, accumulates beyond a threshold value that the plant cells can tolerate, chronic injury occurs. It is estimated that sulphate is about 30 times less toxic than sulphite (Thomas, 1951).

Acute injury is macroscopic necrotic injury to plant tissue variable within hours or days after exposure to short-term (less than 24 h) high concentrations of SO_2. Acute injury to broad leaves takes the form of lesions on both surfaces, usually appearing between veins, and often more prominent toward the petiole and injury is local. The metabolic processes are completely disrupted in the dead or necrotic areas with the surrounding green tissue remaining functional. In some cases, injury can occur on the margins of the leaves. Young leaves rarely display necrotic markings, whereas fully expanded leaves are most sensitive to acute SO_2

injury. The anatomical effects of acute injury on foliage can be seen under the microscope. Initially water-soaked, flaccid areas of diffuse, gayish-green colouration appear and the chlorophyll appears to have diffused from the chloroplasts into the cytoplasm. This is followed by desiccation and shrinkage of the affected cells. The green pigments are decomposed, and the affected cells. The green pigments are decomposed, and the affected leaf area assumes a bleached. ivory, tan, orange-red, reddish-brown, or brown appea . Sulphur dioxide concentrations that caused injury to vegetation in short-term periods are listed in Table 2.

Acute injury to forest trees in the field has been attributed to SO_2 doses of 0.95 ppm for 1 h, 0.55 ppm for 2 h, 0.35 ppm for 4 h and 0.25 ppm for 8 h (Dreisinger, 1965). For acute injury to occur, other environmental and plant factors are important. These includes sunlight, moderate temperature, high relative humidity, adequate soil moisture, and plant genotype and the stage of growth. Katz and McCallum (1939) subjected various plant species to different SO_2 concentrations over a number of exposure periods. The threshold for injury was found to be 0.3 ppm SO_2 for 8 h for western larch (*Larix occidentalis*) and 0.14 ppm SO_2 for 12 h for Douglas fir (*Pseudotsuga taxifolia*). In a 1-hr experimental exposure Zimmerman and Crocker (1934) reported that 0.66 ppm SO_2 injured buck-wheat (*Fagopyrum esculentum*).

TABLE 2

Responses to sulfur dioxide - acute effects

SO_2 conc. (ppm)	Exposure period (h)	Plant response	References
		A-Responses to low SO_2 doses	
0.03	1	Injury to sensitive eastern white pine	Costonis (1971)
0.025	6	Injury to sensitive eastern white pine	Houston (1974)
		B- Responses to medium SO_2 doses	
0.25	1	Injury to eastern white pine	Berry (1967)
0.25	2	Injury to eastern white red and jack pines	Berry (1971)
0.25	2	Injury to Virginia, short leaf, slash,and loblolly pines	Berry (1974)
0.25	4	Injury to broccoli	Tingey *et al.* (1973)
0.66	1	Injury to buckwheat	Zimmerman and Crocker (1934)
0.95	1	Injury to foliage of forest trees	Dreisinger (1965)
0.70	1	Injury to forest trees under sensitive environment	Dreisinger and McGovern (1970)
0.3	8	Injury to weastern larch	Katz and McCallum (1939)
		C-Responses to high SO_2 doses	
2.0	2	Injury to 10 of 87 native desert species	Hill *et al.* (1973)
2.0	4	Injury to 10 weed species	Benedict and Breen (1955)
3.0	1	Injury to *Acacia pruinosa*	O'Conner *et al* (1974).

Acute injury was produced on foliage of trembling aspen (*Populus tremuloides*) in artificial fumigation with 0.35 ppm SO_2 for a period of 3 h. Moderate to severe injury to several Kentucky bluegrass cultivars (*Poa pratensis*) in artificial fumigation of 0.2 ppm SO_2 for 2 h was observed

The acute effects referred to above may be considered responses to medium doses of SO_2. In the literature there are reports of responses of plants to much lower and to substantially higher SO_2 doses. In artificial fumigation experiments SO_2 doses as low as 0.3 ppm for 1 h (Costonis, 1971) have been reported to injure extremely sensitive strains of eastern white pine; and conversely SO_2 doses as high as 2.0 ppm for 2 h (Hill *et al.*, 1973), have been found to be required in order to injure tolerant plant species as *Acacia pruinosa*, several native desert plant species, and several chrysanthemum varieties respectively. Khan (1989) found browning and chlorosis in leaves of egg plant, tomato and okra induced by the SO_2 specially at 0.2 ppm.

Chronic injury is macroscopic chlorotic injury (sometimes changing to necrotic injury) to plant tissue usually developing over a long period of time (from over a day to 1 or more years) from exposure to variable concentration of SO_2. Long term chronic effects on trees are related to variety of SO_2 exposures which includes short-term high concentration, short term and long-term periods of

sublethal concentrations, and even SO_2-free periods when the plant life can recuperate by translocating and assimilating accumulated sulphur. To correlate chronic effects on forest trees with atmospheric SO_2 levels, it is preferrable to use the average concentration for the total period of exposure, rather than the average of SO_2 fumigation periods only. The U.S. Environmental Protection Agency (1973) has concluded that many short-term high concentration episodes were responsible for the injury and growth reductions to occur in the vicinity of an iron ore concentrator that emitted only about 10 tons of SO_2 daily. Although vegetation injury was severe in the immediate vicinity of the plant, SO_2 damage extended for only a little more than 2 km from the source (Guderian and Stratmann, 1962). Linzon (1958a) observed that three large nickel and copper smelters that discharged about 6000 tons of SO_2 daily into the atmosphere, caused severe damage to pine forest with both acute and chronic effects occurring to a distance of about 25 km miles. Beyond this distance the condition of white pine improved rapidly when the concentration of SO_2 were lower than 0.25 ppm. Chemical analyses of vegetation in the area showed the accumulation and build-up of sulphur in foliage in vegetation growing close to SO_2 sources. Both high and low concentration fumigations of SO_2 over varying periods of time contributed to the accumulation of sulphur in conifers which lead to the development of chronic injury in older needles. In

Czechoslovakia occurrence of moderate chronic injury to foliage of spruce trees, under the influence of an average concentration of SO_2 of 0.019 ppm was observed. Epiphytic lichens which are perennial and evergreen are extremely sensitive to SO_2 because they are continually exposed to the gases in a polluted environment. In a study conducted by LeBlanc *et al.* (1972), the number of epiphytes found growing on balsam poplar (*Populus balsamifera*) trees was drastically reduced in zones where the growing season mean levels of SO_2 were over 0.02 ppm and slightly reduced in zones where the mean levels of SO_2 were over 0.01 ppm. Similarly Skye (1964) found that the survival of lichens was less in areas with an annual SO_2 concentration of approximately 0.015 ppm.

Chronic injury appears as a yellowing or chlorosis of the leaf, sometimes from lower to upper surfaces on broad leaves. Occasionally only a bronzing or silvering will occur on the under surface of the leaves. The rate of metabolism is reduced in leaves displaying chronic injury. Chronic injuries develop slowly on coniferous perennial foliage, with the greatest increase injuries in the Sudbury sulphur-fume effects area occurring on the 1-year-old needles of eastern white pine trees. Continued chronic injury to perennial foliage of coniferous trees results in premature needle abscission, reduced radial and volume growth, and early death of the trees. Table 3 lists the SO_2 concentrations causing chronic injuries.

TABLE 3

Responses to sulphur dioxide - chronic effects

SO_2 Conc. (ppm)	Exposure period	Plant response	References
0.07	3 days	Injury to sensitive eastern white pine	Banfield (1972)
0.076	14 days	Injury to barley	Mandl *et al.* (1975)
0.05	24 days	Injury to beech	Keller (1976)
0.067	26 weeks	Depressed yields of ryegrass of 52%	Bell and [illegible] (1973)
0.035	Anuual avr. (5 months growing period)	Injury to eastern white pine in field	Roberts (1976)
0.008	10 year avr. (5 m. growing season)	Slight injury to old foliage of eastern white pine trees in forest	
0.015	Annual avr.	Reduction in lichens and bryophytes in field	Skye (1964)
0.016	Annual avr.	Very low diveristy in lichen and bryophyte species in the field	Gilbert (1969)
0.02	Growing season avr.	Epiphyte species drastically reduced in forest.	Le Blanc *et al.* (1972).

Subtle effects are measured by physiological or biochemical changes, and/or reduction in plant growth or yield in the absence of macroscopic injury. Early investigators studying the effects of SO_2 on vegetation in Europe concluded that invisible injury, or physiological disturbances or effects on the growth or yields of plants could occur in the absence of visible markings (Stoklasa, 1923). This theory was refuted by several investigations carried out in North America in the 1930s (Hill and Thomas 1933; Katz *et al.*, 1939). These investigators found that non-marking concentrations of SO_2 emitted under controlled conditions for long period of time did not affect carbon dioxide assimilation, stomatal behaviour, chemical composition or the rate of growth of the exposed plants. Davis (1972) found no yield loss from soybean unless there were visible manifestation resulting from SO_2 fumigations. Subtle effects without microscopic visible injury to plant tissues, have been reported in other investigations. Bennett and Hill (1974) found that subnecrotic pollutant exposures could repress photosynthetic rates. Table 4 lists the results of experiments conducted to determine the occurrence of subtle effects on plants in the presence of controlled SO_2 doses. Table 5 differentiates the three types of plant effects (acute, chronic and subtle) caused by SO_2.

TABLE 4

Responses to sulfur dioxide - subtle effects

SO_2 Conc. (ppm)	Exposure period	Plant response	References
0.25	1 hr.	2% depression in CO_2 uptake by alfalfa	Bennett and Hill (1974)
0.5	1 hr.	21% Depression in CO_2 uptake by alfalfa	
0.7	1 hr.	Increased amino acids, decreased protein synthesis in bean.	Godzik & Linskens (1974)
0.71	1 hr.	55% inhibition of pollen tube length of lily	Masaru *et al.* (1976)
1.0	30 min	Reduced chlorophyll in cotyledons of red pine	Constantinidou *et al.* (1976)
0.5	2 hr.	Reduced chlorophyll in cotyledons and decreased dry weight of primary needles of red pine	
Ambient air	187 days	Ryegrass grown in water scrubbed air with 98-100% of SO_2 removed had 48% more dry weight than plants grown in polluted air.	Bleasdale(1973).
0.067	26 weeks	Decreased yields of ryegrass by 52% (Chlorosis of leaves but no lesions)	Bell and Clough (1973).
0.05	31-60 days	Decreased photosynthesis in Scot pine	Keller (1976)
0.05	141 days	Decreased pollen germination in white fir.	
0.05	9 months	Increased peroxidase activity in spruce needle homegenates.	

TABLE 5

Differentiation between three types of plant effects caused by SO_2

Acute effects caused by high concentrations of SO_2 during short-term exposure (less than 24 h)	Chronic effects caused by variable conc. of SO_2. Usually over a long period of time.	Subtle effects caused by variable concentrations of SO_2.
Necrotic lesions on foliage of the current year	Chlorotic markings on foliage, sometimes developing to necrosis, on perennial conifers usually on foliage older than current year	No foliar markings
Folair lesions appear within hours or days after exposure	Foliar injury usually develops slowly over a long period of time	May be possible to measure effects on physiological processes photosynthesis, respiration, and transpiration; on biochemical processes-enzyme activities and chemical compositions; on pollen tube elongatin; or on pollen germination
May cause reduction in growth and loss in yield	May cause reduction growth and loss in yield	May cause reduction in growth and loss in yield
Rarely result in plant mortality unless from recurring acute fumigations	May result in plant mortality from slowly developing injuries (especially on perennial conifers and lichens)	No plant mortality

Studies conducted in England report losses in yields of S23 ryegrass (*Lolium perenne*) reported that ryegrass plants exposed to ambient air in one glasshouse weighed between 16 and 57% less than plants grown in similar air in another glasshouse that had 98 to 100% of the SO_2 removed by water scrubbing. Keller (1976) introduced the term 'latent' for subtle effects and reported the results of long-term exposure to a low concentration of 0.05 ppm SO_2. The effects included decreased photosynthesis of Scots pine (*P. sylvestris*), decreased pollen germination in white fir (*Abies alba*), and increased peroxidase activity of needles homogenates of spruce (*Picea excelsa*). Godzik and Linskens (1974) found an increase in the total amount of free amino acids and a concurrent reduction in protein synthesis in primary leaves of Widusa bean (*Phaseolus vulgaris*) when artificially fumigated with 0.70 ppm SO_2. Some of the effects were observed after only 1 h of fumigation and before the occurrence of visible injury. Subtle effects of SO_2 have been reported on pollen tube elongation in vitro studies.

Reinert *et al.* (1980) found that growth of soybean was inhibited by exposure to So_2 at the concentration of 25 ppm, when plants were exposed for 4 h three times per week for 11 weeks. The main effect of SO_2 was a reduction of shoot dry weight at 7th week and total plant growth at 11th week. Kress *et al.* (1986) exposed soybean plants to intermittent SO_2

fumigations (4 h a day, 3 days a week) from shortly after emergence untill maturity. About 7% yield losses due to 0.10 ul/L of SO_2 in intermittent exposures was observed. Foliage of many cultivars of soybean were sensitive to SO_2, but the amount of injury varied with cultivars. The point of attack of sulphur dioxide in grasses (Benedict or Breen, 1955b; Brennan *et al.*, 1970; Bell and Clough, 1973; Cowling and Lockyer, 1976; Bell, 1982) and in pines (Karpen, 1970; Berry, 1971; Roberts, 1976) is the tip of foliage which becomes reddish brown followed by necrosis in contrast to the broad leaved plants where the symptoms may appear anywhere on the leaf.

Susceptibility and resistance of plants to SO_2

Different plant species and varieties, and individuals of the same species, may vary considerably in their sensitivity or tolerance to SO_2. Crop plants are classified into three resistance groups according to their tolerance limits. Clover-type fodder plants were most sensitive to SO_2; wheat, leafy vegetables (excluding cabbage), beans, strawberries, and roses were moderately sensitive; and root crops and cabbage were least sensitive. Ruston (1914) reported that SO_2 caused a marked increased in soil acidity, reducing the number of soil bacteria. Jones Eustace (1952) observed the disappearance of lichens in heavily polluted atmosphere which was another early report of disturbed plant

population due to air pollution. Miller *et al.* (1974) found that foliage of many cultivars of soybean were sensitive to SO_2 but the amount of injury varied with the cultivar. Reinert et al. (1980) found the growth of soybean was inhibited by exposure to 25 ppm of SO_2 when plants were exposed for 4 h, three times per week for 11 weeks. The main effect of SO_2 were a reduction of shoot dry weight at 7th week and total plant growth at 11th week. SO_2 contributed to the reduced growth in soybean in the absence of visible SO_2 injury. Sprugel *et al.* (1980) observed that soybean plant were relatively sensitive to elevated SO_2 concentrations that occurred near a point source. Reinert *et al.* (1982) found that marigold shoot and root dry weights were decreased when plants were exposed to 0.3 ppm SO_2. It reduced dry flower weight too. Sheng *et al.* (1988) exposed soybean cultivars to 0.7 ppm SO_2 which resulted in reduction in net photosynthesis and stomatal conductance and all the cultivars developed typical SO_2 symptoms during or after fumigation.

Physiological and biochemical effects of SO_2 on plants

The manner in which SO_2 affects the metabolism of the plant is not well understood. At the level of the plant, or the plant leaf, the metabolic process of primary interest is photosynthetic carbon dioxide fixation. Since 90 to 95% of the dry weight of plants is derived from this process (Zelitch, 1975), the impairment of photosynthesis has been a popular explanation for plant growth reduction caused by SO_2.

Different plant species have different capacities to remove SO_2 from the surrounding air (Jenes *et al.*, 1976). Lucerne was found to be susceptible to SO_2 only if the stomata were open. This has been confirmed by numerous investigations since then and it is generally accepted that the primary factor controlling SO_2 uptake by plant leaves is the degree of opening of the stomata. Generally, drought is considered to be protective of the plant since the stomata close during this condition. An initial increase in transpiration has been noticed during SO_2 exposure Weigl *et al.*, 1962. Sulphur dioxide may also react with chemical compounds making up the cuticle and thus change the cuticular resistance. It has been suggested that SO_2 stimulates the production of waxes. From scanning electron micrographs it can be seen that SO_2 alters the surface of confier needles. The inhibition of photosynthesis is often regarded as the first stage of SO_2 action on plants. In short-term fumigation the most sensitive lichen species yet studied is *Lobaria pulmonaria*, whose photosynthesis was inhibited after exposure to 0.5 ppm SO_2 for 14 h. SO_2 injury increases respiration rate of the affected plants, the rate of respiration increases initially in pine and spruce plants exposed to SO_2.

Combustion of sulphur-containing fuels yields SO_2 in quantities 40 to 80 times that of SO_3 (U.S. Dept. of Health, Education and Welfare, 1969). Sulphur dioxide is oxidized in

the atmosphere catalytically or photochemically. The photo-oxidation rate of SO_2 in air and sunlight is between 0.1 and 0.2% h. The SO_3 molecules combine with water vapour in the air to form sulphuric acid (H_2SO_4) aerosols. Acid smog injury to vegetation observed in Los Angeles was described by Thomas *et al.* (1952). Spots were formed on the upper surface of the leaves which later extended throughout the leaf. In the Scandinavian countries, the north-eastern United States, acid rain has been found to have had serious implications with respect to increasing the acidity of lakes and reducing fish populations. It has been postulated that decreased productivity of forests in susceptible region in Sweden is attributable to natural rainfall acidified by industrial emissions. Wood and Bormann (1974, 1975) and Wood (1976) reported foliar tissue damage of yellow birch (*Betula alleghaniensis*) seedlings, using artificial acid mist of pH 3.0, and significant growth decrease at pH 2.3; increased foliar leaching of potassium, magnesium, and calcium from sugar maple (*Acer saccharum*) and pinto bean seedling using artificial acid mists of pH 4.0, and tissue damage at pH 3.0; and a 20% increase in growth of eastern white pine seedling subjected to artificial acid rain at a pH of 2.3 compared to rains with higher pH. However, soil acidity increased, and magnesium, calcium and potassium cations were leached from the soil at the low pHs.

Sulphur dioxide after its absorption through stomata accumulates into intracellular spaces. A large amount of SO_2 can be absorbed by the cells of the leaf without any injury if the absorption is slow and the concentration of the gas is low. However, SO_2 accumulates faster than it can be oxidised and assimilated thus, exceeding the threshold level, and a phytotoxic concentration presumably develops in the intercellular spaces of the leaves, causing injury (Katz, 1952; Thomas, 1961). The excess SO_2, accumulated in the intercellular spaces, comes in contact with the water bathed cell walls of the surrounding tissue to form sulphurous acid and sulphates, leading to plasmolysis and collapse of the cell (Brandt and Heck, 1968). Haselhoff and Lindau (1903) believed that the gas reacts with aldehydes and sugars to form secondary products which slowly release sulphurous or sulphuric acid causing injury to the cell. However, the idea could not last long, since the leaves are more sensitive to SO_2 in morning when the sugar content is low (Treshow and Pack 1970). SO_2 inactivates iron in the chloroplast causing interference with its catalytic properties in assimilation. This promotes secondary processes and break down the chlorophyll, ultimately killing the cells. Dorries (1932) observed that the absorbed acidity could decomposed chlorophyll, liberating magnesium and forming pheophytin; which may lead to chlorosis and reduced photosynthesis. According to Thomas *et al.* (1943), the toxicity of sulphurous

acid is related primarily to its reducing properties rather than to its acidity because it is 30 folds more toxic than sulphuric acid. Excess of sulphate may interfere with ion absorption, leading to disruption in nutrient balance. Bleasdale (1952) found that normally an equilibrium is maintained between sulphydryl group and more oxidized sulphur compounds such as sulphites. Any imbalance in this equilibrium, as might be caused by an excess of oxidized sulphur compounds, would upset sulphur utilization and protein synthesis. Sulphur dioxide would reduce the sulphites to sulphydryl, causing an accumulation of the latter and disturbing the ratio between sulphydryl to oxidized sulphur compounds. The sulphydryl compounds thus becomes a products of a disturbed sulphur metabolism, and the tolerance of plants to SO_2 may be a function of the stability of sulphydryl (Dekok *et al.*, 1982). Bennett *et al.* (1973) observed that rate of uptake of gaseous pollutants into a leaf depends on several physical factors. Before being capable to cause injury within the plant cell, the pollutant has first to enter the solution in the extra-cellular water contained in the cell wall. The simple solubility in water is therefore, likely to assume importance while considering two pollutants which differ markedly in solubility and are taken up at different rates by the plants (Law and Mansfield, 1982). MacCormick (1968) observed that the effect of SO_2 in "daily cycle" is more in the morning (7-10 A.M.) and in the

evening (7-10 P.M.). This diurnal change in sensitivity corresponds to the diurnal changes of stomatal aperture in a number of plants. During the "seasonal cycle" the effect of SO_2 is more during winter than in summer (MacCormick, 1968). Experiments under two light regimes depicting winter and summer have proved that 50% reduction in shoot dry weight was obvious when SO_2 fumigation accompanied winter lights (Davies, 1980).

Nematode Parasites of Pulses

Pulses has been found to be attacked by a number of nematodes and their pathogenicity have been proved (Gupta *et al.*, 1986; Kinloch, 1980;2 Mujib *et al.*, 1988; Patel *et al.*, 1988: Chavda *et al.*, 1988; Nath *et al.*, 1979; Mani *et al.*, 1984; Rodriguez-Kabana *et al.*, 1981; Herman *et al.*, 1988; Routary *et al.*, 1986; Wrather *et al.*, 1988; Kinloch *et al.*, 1987; Sobun *et al.*, 1979; Dhanger and Gupta 1983; Srivastava *et al.*, 1974, Upadhyay and Swarup, 1981; Singh and Gill, 1990). The important nematodes which are usually found in association with the roots of soybean and check-pea are *Heterodera glycines*, *Pratylenchus brachyurus*, *Tylenchorhynchus* spp., *Tylenchus* spp., *Helicotylenchus* spp., *Hirschmaniella* spp., *Rotylenchulus reniformis*, and *Meloidogyne* spp.

Kinloch (1980) reported that the most widespread and damaging nematodes in soybean production are *Meloidogyne*

incognita (Kofoid & White) Chitwood and soybean cyst nematode, *Heterodera glycines* Ichinode. Rodriguez-Kabana *et al.* (1981) observed that *M. arenaria* (Neal) Chitwood and *M. incognita* prevalent in sandy soils can severely limit soybean, *Glycine max* (L.) Merr. yields. Wrather and Anand (1988) found that soybean seedling growth was inhibited by *H. glycines* infection and soybean sensitivity to the nematode seemed to diminish with the age of plant. Kinloch *et al.* (1987) conducted a field trail with 39 soybean cultivars and 5 breeding lines at sites infested with *M. arenaria*. Yields of all cultivars were too low to justify their planting in sites heavily infested with *M. arenaria*. Herman *et al.*, (1988) observed that *M. incognita* suppressed shoot growth of soybean even that which was claimed to be resistant to nematode.

Mani and Sethi (1984) studied the pathogenicity of *M. incognita* on chick-pea cultivar Pusa-209 with 5 inoculum levels, namely, 0.5, 1.0, 2.0, 4.0, and 8.0 larvae per gram of soil. They found that there was a progressive decrease in plant growth as the inoculum level of the nematode increased. Nath *et al.* (1979) studied the effect of *M. incognita* on chick-pea. They found that increase in the level of larval inoculum of the nematode resulted in the proportional decrease in plant growth, flowering, fruiting and bacterial nodulation in chick-pea.

Root-knot nematodes are one of the most destructive plant parasitic nematodes with a wide host range and thriving in a greatly diverse habitats. Gupta *et al.* (1986) screened 219 varieties / lines of mung bean for their susceptibility to root-knot nematode, *M. javanica* and found none as resistant. Mujib *et al.* (1988) studied effect of *M. incognita* on carpet legume (*Dolichos lablab* v. *lignosus*). They observed an decrease in the length, fresh weight of shoot and root as the inoculum density of *M. incognita* increased and significant reduction in growth occurred with 1000 nematode per 500 cc soil. Out of 60 cowpea lines/germplasm screened by patel *et al.* (1988) for pathogenicity of mixed population of *M. javanica* and *M. incognita* none was found to be resistant. Chavda *et al.* (1988) studied reaction of 27 varieties/lines of greengram to a mixed population of *M. incognita* and *M. javanica* and found that all the tested cultivars were either moderately or highly susceptible.

Pulses and Soil-borne Fungal Pathogens

Pulses are known to suffer from number of soil micro-organisms among which fungal pathogens causing wilt and root-rot are of the main limiting factors in their production. The most important ones causing severe damage to pulses are *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Chattopadhyay, *Rhizoctonia bataticola* (Taub.) Butl. (=*Sclerotium bataticola* Taub, *Macrophomina phaseolina* (Tassi) Goid.), *Fusarium solani*

f.sp. *pisi*, *F. eumartii*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Operculella padwickii*, *Verticillium albo-atrum*, and *Botrytis cinera*.

Mani and Sethi (1985) founded five pathogenic fungi viz. *Fusarium oxysporum* f.sp. *ciceris*, *F. solani* f.sp. *pisi*, *Phythium ultimum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* in association with root-rots and wilt of chickpea. Of these, *F. solani* is reported to cause appreciable loss to chick-pea under Delhi conditions. Initially the diseased plants showed yellowing of leaves and the side shoot started drying and showed characteristic brown discoloration. Uprooting of such plants, showed brown coloured, irregular to round lesions on the collar regions. In advanced stages, the collar got constrained and the plant tappled down. Bhatti *et al.* (1987) observed that isolates from wilted *Cicer arietinum* plants included *Fusarium* spp. (42%), *R. solani* (28%). *Macrophomina phaseolina* (17%), and *V. albo-atrum* (4%). Westerlaud *et al.* (1974) observed that *F. solani* f.sp. *pisi*, and *F. oxysporum* f.sp. *ciceris* causes similar yellowing and wilting of the chickpea shoots, but the former caused distinctive black root lesions and the latter, vascular discoloration extending to the top of the shoot. They observed that *F. oxysporum* f.sp. *ciceris* may require wounding for efficient infection, but *F. solani* f.sp. *pisi* does not. It was found that *F. oxysporum* f. sp. *ciceris* was

not seed-borne. There were evidences which showed *F. solani* f. sp. *pisi* was seed borne.

Wilt of chick-pea which is caused by several soil-borne fungal pathogens including *F. oxysporum* f. sp. *ciceris*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* (*Macrophomina phaseolina*), and *Opercalella padwicki* has been reported by Kotasthane *et al.* (1980) to be a limiting factor in its production in Madhya Pradesh. Mortality in the early seedling stage was chiefly due to *S. rolfsii* while at flowering *F. oxysporum* f.sp. *ciceris* was the major pathogen. Alvarez and Bringer (1987) isolated *F. solani* from chick-pea plants showing reduced growth, leaf yellowing, defoliation and premature death. There were collar and root lesions, but root vascular discoloration. Trapero-Casas and Jimenez-Diaz (1985) in a disease survey in 1979-81 observed that chick-peas in southern Spain were severely affected by a wilt and root-rot complex. Symptoms included vascular wilt or yellowing, non-vascular yellowing, collar and root-rots or cortical collar and root necrosis, and yellow stunt. They found that *F. oxysporum*, *F. solani* and *M. phaseolina* were associated with the wilt and root-rot complex. Salmon-pigmented isolates of *F. oxysporum* induced vascular wilt or yellowing, and reddish-pigmented isolates induced non-vascular yellowing and cortical collar and root necrosis. *F. solani* and *F. eumartii* induced non-vascular yellowing and black collar and root

rot, *M. phaseolina* induced dry collar and root-rot. Simay (1989) detected *Botrytis cinerea* from chick-pea seedlings showing pod and seedling-rot symptoms. Wilt of chick-pea caused by *F. oxysporum* f. sp. *ciceris* for the first time in India was observed in 1940 by Padwick (1941), which causes about 10% losses in yield annually (Singh and Dahiya, 1973).

Murumkar and Chavan (1985) observed that infection of chick-pea by *F. oxysporum* f.sp. *ciceris* resulted in reduction in chlorophyll and increase in organic acids, polyphenols and carbohydrates. Toxins isolated from culture filtrate of chick-pea wilt pathogens *F. oxysporum* f.sp. *ciceris* by Kaur *et al.* (1987), caused browning and inhibition of callus growth, and reduced free proline and soluble protein content of cell. Wilt resistant varieties were less sensitive to toxin. Toxin did not result in bursting of protoplast. Primary site of action of toxins was inhibition of RNA synthesis. Murthy and Bazyaraji (1980) observed that the root nd shoot protein of resistant varieties contained higher amounts of total alkaloids than the susceptible cultivar. Alkaloids were fungitoxic and accumulated at the site of wound.

F. oxysporum f. sp. *ciceris* and, at a lower rate, *F. solani* f.sp. *pisi* were detected inside chick-pea seeds. *F. oxysporum* f. sp. *ciceris* reaches seeds by systemic pathway varying for each studied line, while *F. solani* f.sp. *pisi*

was traced only to the stem and plant collar (Conci *et al.*, 1985).Sharma and Gupta (1986) noted that chlamydospores of *F. oxysporum* f.sp. *ciceris* remained viable throughout the high temperature of the summer month during the monocropping period in naturally infected roots of *Cicer arietinum* at soil depths of 5,10 and 15 cm. The fungi did not survive in roots placed on the soil surface.

The ubiquitous sclerotial fungus *Rhizoctonia bataticola* in its pycnidial state is known as *Macrophomina phaseolina*. The fungus causes complex disease syndromes like charcoal rot of stem, root-rot, seedling blight, foliage blight, tuber decay, dry rot, fruit rot, pod nd seed rot in several crops. In absence of host plant, it survives or over seasons predominantly as small black sclerotia in diseased plant parts or in soil. *R. bataticola* from different host species differ in their morphological and cultural characters and even differences occur in the isolates from various parts of the same host (Jain *et al.*, 1973; Dhingra and Sinclair, 1973; Ghosh and Sen, 1973; Grover and Sakhuja, 1981; Hooda and Grover, 1982; Byadgi and Hedge, 1985). *M. phaseolina* is an important pathogen of many hosts like lima bean (Andrus, 1938), Jute (Varada Rajan and Patel, 1946), black gram (Tarr, 1954), sesame (Meiri and Solel, 1963), soybean (Gangopadhyay *et al.*, 1970) and sweet potato (Stevens *et al.*, 1987). The location of pathogen in the seeds decides the disease

severity. Firm establishment of the pathogen within the seed tissue is pre-requisite for its effective transmission.

Widespread losses at different stages of soybean growth are caused by species of *Colletotrichum*, *Rhizoctonia*, *Sclerotium*, *Macrophomina*, and *Fusarium* (Perera, 1985). Sharma and Bhowmik (1987) observed that *M. phaseolina* (=*R. bataticola*) infection caused both quantitative and qualitative damage to groundnut seeds. It resulted in characteristic discoloration of pod and kernel, and a marked reduction in pod and kernel yield, shelling percentage and oil content. Prinsloo (1986) noted that *M. phaseolina* caused symptom of black root-rot on chicory only when roots were tooth-pick-inoculated in the laboratory. *M. phaseolina* caused symptoms only when the roots were injured by *Thielaviopsis basicola*. All the cotton cultivars test by Lee *et al.* (1986) were susceptible to isolates of *M. phaseolina* from *Phaseolus vulgaris*, cotton and groundnut. The groundnut isolate (M14) was the most virulent on cotton and mechanical wounds were not necessary for infection. The stele portion of the tap root and pith in the lower portion of the main stem and root were discolored, varying from rust-brown to black. Sclortia of the pathogen were found in the pith and the organism could be isolated from the stele. All the soybean cultivars tested by Garica *et al.* (1985) were found to be susceptible to *Sclerotium rolfsii*, *Fusarium* spp., *R. solani*, and *M.*

phaseolina. Anahosus and Naik (1986) observed that *M. phaseolina* colonizes in root and stalk of sorghum leading to disintegration of their tissues.

Isolates of *M. phaseolina* obtained from different plant species differed in cultural characters, growth rate, morphology of the sclerotia, pycinidia and pycinidiospores, and virulence. Bean, chick-pea and cowpea isolates were the most virulent (Byadgi and Hegde, 1985). Raut and Ingle (1989) observed variation between the isolates of *R. batatico* collected from different cultivated crops belonging to seven families. Differences were abundant even between the isolates of crops belonging to same family. Chitima *et al.* (1987) also observed differences in morphology, growth rate and pathogenicity among isolates of *M. phaseolina* and also to some extent between individual conidia originating from a single pycnidium.

Prasad (1986) concluded that incidence of root and stem rot disease of jute caused by *M. phaseolina* was inversely proportional to K levels. Maximum disease was observed at 0 Kg Potash/ha. and minimum of 70 Kg potash/ha. In pot culture experiments conducted by Sharma and Chaudhary (1985) it was observed that application of 120, 80 and 40 Kg/ha. of N, P2 O_5 and K_2 O respectively were the best for the reducing mortality of cauliflower seeding suffering from wilt and root rot caused by *R. solani* and *R. bataticola* (*M. phaseolina*).

Losses caused by soil-borne fungi, particularly *M. phaseolina* and *F. oxysporum* f. sp. *vasinfectum*, were estimated at cost $ 481593 over 27000 ha. of cotton in 1982 (Delgado and Agurto, 1984).

Interaction of Sulphur Dioxide and Plant Parasites

Effects of SO_2 on the relationship between plant host and various plant pathogens have been examined both in ambient conditions and artificial treatments. Hegale (1973, 1982) Treshow, (1965) summarized the interactions between several air pollutants including sulphur dioxide, and host-parasite relationships.

There are a number of possible host-parasite interaction effects that may occur when a pollutant is introduced into a particular habitat. The severity of the parasitism may be increased or decreased as a result of the action of the pollutant on the virulence of the parasite or on the susceptibility of the host.

Fungal Plant Pathogens and Sulphur Dioxide

Effects of SO_2 on fungal disease of plants are summarised in Table 6. Fungi may be affected by SO_2 directly or indirectly through some effect upon living or dead plant tissues, soil, or water. Sulphur dioxide results in accumulation of sulphur, increased acidity and other alterations in plant physiology and biochemistry.

TABLE 6

Interaction of sulphur dioxide and plant pathogenic fungi

Fungal plant pathogens	Host	Dose	Effect	Reference
Puccinia graminis	Wheat	Ambient	Less disease incidence	Johanson,1954
Puccinia sp.	Trees	Ambient	Decrease in disease incidence	Scheffer and Hedgcock, 1955
Hypodermella sp.	-	Ambient	Decrease in disease incidence	Scheffer and Hedgcock, 1955
Lophodermium piceae	-	Ambient	Increase in disease incidence	Kundela and Novakova. 1962
Hysterium pulicare	Alder and Brich	Ambient	Decrease in disease incidence	Skye, 19[illegible]
Melampsora sp.	-	Ambient	Increase in disease incidence	Scheffer and Hedgcock, 1955
Trametes serialis and *T. heteromopha*	Trees	Ambient	Increase in disease incidence	Jancarik, 1961
Rhizosphaera kalkhoffii	Red pine	Ambient	Increase in disease incidence	Chiba and Tanaka, 1968
Rhytisma ocerinum	Sycamarea	Ambient	Decrease in disease incidence	Bevan and Greenhalgh, 1976
Cronatrium sp.	Trees	Ambient	Decrease in disease incidence	Linzon, 1958; Scheffer and Hedgcock, 1955
Puccinia striiformis	-	Ambient	Decrease in germination of uredospores	Sharp, 1967
Armillaria mellea	Trees	Ambient	Increase in disease incidence	Donaubauer, 1968 Jancarik, 1961; Kundela and Novakova, 1962
Coleosporium sp.	Trees	Ambient	Decrease in disease incidence	Linzon, 1958; Scheffer and Hedgcock, 1955

Table 6 Contd...

Fungal plant pathogens	Host	Dose	Effect	Reference
Uromyces phaseoli	Bean	0.13 ppm for 24 h/d (on 8 days before or 7 days after inoculation)	Decrease in pustule number, spore size spare size and germination	Weidensaul and Darling, 1979
Scirrhia acicola	Scots pine	0.20 ppm for 6 h 5 days after inoculation	Increase in lesion number	Weidensaul and Darling, 1979
S. acicola	Agar medium	1.0 ppm for 4 h	Normal growth	Ham, 1971
Puccinia graminis	Wheat	0.10 ppm for 100 h from 2 days from inoculation	Decrease in lesion number	Laurence *et al.*, 1979
Alternaria sp.	-	50 ppm for 2 minutes at moist condition of 100 ppm for 24 minutes at 98% RH	60% decrease in spore germination	Couey, 1965
Diplocarpon rosae	Rose	0.01 and 0.04 ppm for 2 days after inoculation	Diseased leaflet area slightly increased at at 0.01 ppm more than the 0.04 ppm	Saunders, 1966
Penicillium sp.	-	90 ppm SO_2 in nutrient solution	Slight stimulation in growth	Saunders, 1966
Helmintho sporium maydis	Maize	0.15 ppm for 14 h/d 8 days before inoculation	Decrease in lesion number	Laurence *et al.*, 1979
Microsphaera alni	Lilac	0.30-0.40 ppm for 24-72	Decrease in conidial	Hibben and

Generally, excess of SO_2 in the atmosphere tends to decrease the severity or incidence of host-parasite relationship. Rust diseases appear to be particularly sensitive to SO_2. Johanson (1954) reported less wheat stem rust caused by *Puccinia graminis* in an industrialized area of Sweden than in non industrialized areas. Scheffer and Hedgcock (1955) found decrease of a number of rust diseases caused by species of *Cronartium*, *Coleosporium*, *Melampsora*, and *Peridermium*, where trees were injured by SO_2. Weinstein *et al.* (1975) found that SO_2 reduced the incidence and severity of bean rust disease caused by *Uromyces phaseoli* on pinto bean. Continuous exposure to 0.13 ppm of SO_2 on the 8 days before inoculation or on the 7 days after inoculation caused foliar injury and decreased the number of pustules and the size and percentage germination of spores produced on exposed leaves. In general, obligate fungal plant pathogens are more sensitive to SO_2 as compared to other fungal plant pathogens. Fungal diseases on plant foliage have been reported to be reduced in incidence by atmospheric SO_2 in the vicinity of industries in several countries. These leaf fungi includes *Hypodermella laricis*, *Lophodermium pinastri*, *Hypodermella* sp. (Scheffer and Hedgcock, 1955), *Lophodermium juniperi*, *Rhytisma acerinum* (Air pollution, 1968), *Hysterium pulicare* (Skye, 1968), and *Venturia inaequalis* (Przybylski, 1967). Kock (1935) reported the absence of oak powdery mildew caused by *Microsphaera alni* near a paper mill in Austria.

Saunders (1966) observed that rose black spot disease caused by *Diplocarpon rosae* was rarely present in areas where the daily average SO_2 concentration was greater than 0.04 PPM. Khan and Kulshrestha (1991) studied effect of SO_2 on conidial germination of eight powdery mildew-fungi species viz., *Sphaerotheca fuliginea* (Schlecht.) Poll., *S. cassiae* Pandotra & Ganguly, *Erysiphe cichoracearum* DC., *E. trifolii* Grev., *E. pisi* DC., *Microsphaera alphitoides* Griff & Maubl. f. sp. *zizyphi* and *Phyllactinia dalbergiae* Pr ioz. They observed that all the conidia tested were sensitive to SO_2 and its germination was increasingly inhibited with an increase in the concentration of SO_2.

Effect of SO_2 on parasitism by non-obligate fungal pathogens show cases of inhibition, stimulation, or no response. Exposures that decreased parasitism of bean by *Uromyces phaseoli* did not affect parasitism of tomato leaves by *Alternaria solani*. Laurence *et al.* (1979) observed decrease in number of *Helminthosporum maydis* lesion by 38% when maize plants were exposed to SO_2 on the 8 days before inoculation (0.15 ppm for 14 h daily) if the exposure occurred on the 8 days before and on the 2 days after inoculations, the number of lesions decreased by only 13-16%. Spores of most fungi tested appear to be very resistant to direct exposure to SO_2 and moist spores apparently are more sensitive to than the dry ones. Couey and Uota (1961)

observed that only 20% of the conidia of *Botrytis cinerea* germinated when they were exposed to 36 ppm SO_2 for about 1/2 h. But Hibben (1966) observed no effect on germination of spores of 10 saprophytic and parasitic fungi when they were exposed on agar to 10 ppm SO_2 for 1-6 h. Couey (1965) found that germination of wet conidia of *Alternaria* sp. decreased 60% from exposure to 50 ppm SO_2 for 24 min but 100 ppm SO_2 was required to produce a similar decrease in the dry spores at 98% RH. Conidia of *Diplocarpon rosae* germinated abnormally and hyphal growth was reduced in aqueous sulfite solutions that contained the equivalent of more than 35 ppm SO_2. Spores of other fungi such as *Aspergillus niger* and *Alternaria brassicicola* were also affected but were more resistant than *D. rosae* (Saunders, 1966). Germination of *Scirrhia acicola* conidia suspended in water was not affected by exposure to 0.90 ppm SO_2 for 6 h (Ham, 1971). Khan and Kulshrestha (1991) found that SO_2 exposure inhibited conidial germination of a number of powdery mildews. SO_2 concentration and exposure duration were determinants of inhibition.

Some researchers have found that SO_2 can increase the severity of foliar diseases. Tanaka *et al.* (1982) reported that sooty leaf mold disease, *Rhizosphaera klkhoffii*, was more severe on *Pinus densiflora* trees that were inoculated and transplanted to areas with high concentrations of atmospheric SO_2 than on trees in areas with no air pollution. Weidensaul and Darling (1979) observed more

lesions of *Scirrhia acicola* on needles of scots pine seedings when they were exposed to 0.20 ppm SO_2 at 5 days after inoculation than on unexposed plants. The effect was similar, but not significant, when the 6 h. exposure ended 30 min. before inoculation.

Plant Parasitic Nematodes

The effect of sulphur dioxide on host-parasite interactions involving root-knot nematodes, has gained little study. But, there are few evidences indicating the effect of air pollution on plant parasitic nematodes (Table 7). Bassus (1968) found occurrence of fewer numbers but a greater variety of nematode species in soils where trees were damaged by SO_2. Khan (1989) studied the effect of SO_2, O_3 and SO_2-O_3 mixture of different concentrations on tomato plants inoculated with *M. incognita* race 1. He observed that SO_2 and O_3 (both particularly at 0.2 ppm) and *M. incognita*, acting alone significantly reduced plant growth and yield parameters and leaf pigment content of tomato. Synergism was observed when plants were exposed to 0.2 ppm SO_2 and 0.1 or 0.2 ppm O_3 and greater reduction was observed than the sum of their individual effects. SO_2 and O_3 also individually acted synergistically with the nematode and caused greater reduction in growth parameters than the reduction in nematode inoculated unexposed plants or uninoculated exposed plants. When tomato plants inoculated with *M. incognita* race 1 were

TABLE-7

Effect of air pollution on plant parasitic nematodes

Treatment	Nematode	Host	Effect	References
SO_2	*Pratylenchus penetrans*	Soyabeen	Enhanced reproduction of nematode	Weber *et al.*, 1979
SO_2	*Meleidogyne incognita* Race 1	Tomato cv. Pusa Ruby	SO_2 interacted synergistically with the nematode	Khan, 1989
SO_2	*M. incognita* & *M. javanica*	Lentil, chickpea	Root galling suppressed	Singh, 1989
O_3 and $SO_2 + O_3$	*Heterdora glycines*	Soyabeen	Inhibition in prooucti[illegible] and development of nematode	Weber *al.*, '7-
O_3 and $SO_2 + O_3$	*Heterodera glycines*+*Rhizobium*	Soyabeen	Severe inhibition in nodulation	Weber *et al.*, 1979
O_3 and $SO_2 + O_3$	*Belonolaimus longicaudatus*	Soybeen	Unaffected	Weber *et al.*, 1979
O_3 and $SO_2 + O_3$	*Paratrichodorus minor*	Soybeen	Reproduction and development of nematode inhibited	Weber *et al.*, 1979
O_3 and $SO_2 + O_3$	*B. longicaudatus* +*Rhizobium*	Soyabean	Inhibition in number of nodules	Weber *et al.*, 1979
O_3 and $SO_2 + O_3$	*Aphelenchoides fragariae*	Begonia	Decreased folior injury by nematode	Weber *et al.*, 1979
0.2 ug O_3/ lit., 0.2/ ug SO_2/lit. singly or in combination	*P. penetrans*	Tomato	Synergistic interaction	Shew *et al.*, 1982
0.2 ug O_3/L + 0.8 ug SO_2/L	*P. penetrans*	Tomato	Antagonistic interaction	Shew *et al.*, 1982
80 ppm O_3	*M. hapla*	Tobacco	Increase in disease	Bisessar and Plamer, 1984

Table 7 Contd..

Treatment	Nematode	Host	Effect	References
0.2 ug O_3/L + 0.8 ug SO_2 L	*P. penetrans*	Tomato	Antagonistic interaction	Shew *et al.*, 1982
80 ppm O_3 (ambient)	*M. hapla*	Tobacco	Increase in disease incidence	Bisessar and Plamer 1984
Simulated acid rain of pH 6.0 or 3.2, 3 times weekly	*M. hapla*	Kidney bean	Decreased nematode infection of pH 3.2	Shriner, 1978
O_3	*M. incognita*	Lentil	Root galling suppressed	Singh, 1[illegible]
SO_3+O_3	*M. incognita* Race 1	Tomato	Growth of nematode reduced	Khan, 1989
SO_2+O_3	*M. incognita*	Chickpea	Juvenile hatching suppressed	Singh, 1989
Acid rain at pH 6.6 before inoculation	*M. incognita* Race 1	Tomato	Root penetration and reproduction increased significantly	Khan, 1989
Acid rain at pH 3.2 after inoculation	*M. incognita* Race 1	Tomato	Root penetration and reproduction decreased significantly	Khan, 1989
Acid rain at pH 5 and 6	*M. incognita* *M. javanica*	Lentil chickpea	Juvenile hatching suppressed	Singh, 1989

exposed to SO_2 + O_3 mixture at 0.2 + 0.2 ppm or 0.2 + 0.1 ppm of each pollutant respectively, synergistic reduction in growth of nematode was observed. Hatching of juveniles of *M. incognita* and *M. javanica* was suppressed by exposing to SO_2, O_3 and their mixture. Higher concentration of pollutants were more suppressive than the lower concentrations, and the effect of pollutant mixture was greater than their individual effects. *M. incognita* was more sensitive to pollutant mixture than *M. javanica*. Juvenile hatching of *M. incognita* and *M. javanica* were adversely affected by the acidity of the media and decreased with the increasing acidity. At pH 6, greater suppression in juvenile hatching of *M. incognita* was observed whereas in acidic range (from pH 5 onwards) the suppression in juvenile hatching of *M. javanica* was more than *M. incognita* (Singh, 1989). Khan (1989) observed a synergistic interaction between *M. incognita* race 1 and simulated acid rain at pH 3.2 on tomato plants. They caused greater reduction on tomato plants than the sum of reductions induced by each individually. Root penetration and production of *M. incognita* race 1 and disease intensity were significantly increased at pH 6.6, but at pH 3.2 significant decrease were recorded.

Weber *et al.* (1979) observed that non-injurious doses of SO_2 failed to affect adversely four nematode parasites of soybean viz., *Pratylenchus penetrans*, *Belonolaimus*

longicaudatus, *Heterodera glycines*, and *Paratrichodorus minor*, or one nematode parasite in leaves of begonia, *Aphelenchoides fragariae*. However, reproduction of *P. penetrans* was increased when soybean plants were exposed to SO_2 on the 4 days before inoculation and on 3 days per week for 13 weeks after inoculation (0.25 ppm for 4 h daily). This dose of SO_2 was not enough to injure leave or to affect plant growth. Exposure of soybean plants to SO_2 enhanced the reproduction of *P. penetrans* compared with that in plants exposed to the charcoal filtered air control. Brewer (1979) observed that reproduction of *P. penetrans* on tomato was slightly suppressed by O_3 at 0.2 ppm but not by SO_2 at 0.80 ppm (3 h/day on 2 days per week, for 8 weeks). However, the mixture of 0.20 ppm of O_3 and 0.80 ppm of SO_2 caused increased reproduction of *P. penetrans*. Shew *et al.* (1982) conducted a series of experiments to understand the possible interaction of *P. penetrans* and O_3 and SO_2 on tomato plants. Tomato plants cultivar 'Walter' were inoculated with initial population densities of *P. penetrans* ranging 0-4000 nematodes per pot and were repeatedly exposed for 3 h to O_3 doses ranging 0.0 - 0.4 ul/l of air (1 ul O_3/l of air = 1.960 ug O_3/m^3 of air). In another experiments, tomato plants, uninoculated or inoculated with *P. penetrans* were exposed (4 h per exposure) 15 times to 0.2 ul O_3/l of air, or 0.2 ul SO_2/l (1 ul SO_2/l of air = 2620 ugSO_2/m^3 of air), or both, or were exposed (3 h per exposure) to 0.2 ul O_3 /l of air or

0.8 ulSO_2/l of air or both. Exposures to charcoal-filtered air served as control. Decrease in dry weights of plant parts excised from tomato plants exposed to 0.2 ulO_3/l of air added to the decrease in dry weight, caused by exposure to SO_2 at 0.2 ul/l of air adequately predicated the decrease in dry weight of tomato plants caused by exposure to 0.2 ulO_3 + 0.2 ulSO_2 per liter of air. When 0.2 ulO_3 and 0.8 ulSO_2 per liter of air were present in mixture, they acted antagonistically and caused less change in leaf and shoot dry weight than could be predicated by the main affects of O_3 and SO_2.

Plant Pathogenic and Nodule-forming Bacteria

Laurence and Aluiso (1981) observed that SO_2 effects the parasitism of bacteria. Table 8 shows the influence of SO_2 on some plant bacterial pathogens. It has been observed that *Corynebacterium nebraskense* was inhibited when maize plants were exposed continuously to 0.20 ppm SO_2 for 24 h/day, 5 days before inoculation, 2 days after inoculation and both pre and post inoculations. However, maximum inhibition of bacteria occurred at 2 days post-inoculation exposure. They also observed that *Xanthomonas phaseoli* was inhibited when soybean plants were exposed the 0-10 ppm SO_2 for 24 h 5 days before inoculation, 5 days after inoculation and both 5 days before and after inoculation exposures.

TABLE 8

Interaction of sulphur dioxide (SO_2) and plant pathogenic bacteria and viruses.

Treatment	Bacteria/Viruses	Host	Effect	Reference
0.20 ppm for 24 h/day 5 days before or 2 days after inoculation or both	*Corynebacterium nebraskense*	Maize	Inhibitory	Laurence and Aluisio, 1981
0.10 ppm for 24 h/day for 5 days before or 5 days after inoculation	*Xanthomonas phaseoli*	Soybean	Inhibitory	Laurence and Aluisio, 1981
0.10 ppm for 24 h for 7 days	Southern bean mosaic virus	Bean	Increase in virus concentration	Laurence *et al.*, 198
0.10 ppm for 24 h for 7 days	Maize dwarf mosaic virus	Maize	Increase in virus concentration, infection and severity of symptoms	Laurence *et al.*, 198

Sulphur dioxide increases the soil acidity which can conceivably alter the progress of plant-parasite interactions. Shriner (1974) found that kidney been and soybean exposed over 8 weeks in the field or 5 weeks in the greenhouse to pH 3.2 developed an average of 75% fewer nodules than plants exposed to pH 6 treatments. Overall, 74% of the plants that failed to nodule at all had received pH 3.2 treatments. In a time-related study, nodulation was inhibited most by daily exposure to pH 3.2 treatments applied 15-21 days after plants were inoculated with *Rhizobium*.

Interaction of Sulphur Dioxide with other Air Pollutants

In nature, plants are rarely exposed to the influence of only one air pollutants. Before 1966 some observations and experiments had indicated that air pollutant mixture might influence plants differently from the action of a single pollutant.

Menser and Heggestad (1966) found that three varieties of tobacco plant suffered from 25 to 38% leaf damage upon exposure to a combination of 0.24 ppm SO_2 and 0.027 ppm O_3 for 2 h., whereas either pollutant alone at approximately the same concentration and for the same time period caused no injury to any of the three tobacco varieties. They suggested that the injury to the tobacco plants was caused by a synergistic effect of SO_2 and O_3. The potential harm that may

be caused by mixture of low levels of SO_2 and NO_2 was demonstrated by Tingey *et al.* (1971). A gaseous mixture of 0.10 ppm SO_2 and 0.10 ppm NO_2 caused syngergistic effect with greater than 5% leaf injury being induced on five of six plant species treated in 4 h exposure periods. Bel W-3 tobacco plant displayed 9% leaf injury after a 4-h exposure to a mixture of 0.05 ppm SO_2 and 0.10 ppm NO_2. Tingey *et al.* (1973b) found that foliar injury on broccoli showed an additive response to a mixture of 0.25 ppm SO_2 and 0.10 ppm O_3 for 4 h, whereas Bel W-3 tobacco showed a synergistic response under the same regime. Low concentrations of SO_2 and/or O_3 to injured foliage of peanut plants in controlled experiments. The response to a mixture of the two gases was synergistic. Either 0.35 ppm SO_2 alone for 3 h or 0.05 ppm O_3 alone for 3 h injures the foliage of trembling aspen, a combination of 0.20 ppm SO_2 and 0.05 ppm O_3 for 3 h causes more injury than the sum of the individual pollutants. White *et al* (1974) reported a synergistic response of alfalfa to a mixture of 0.15 ppm SO_2 and 0.15 ppm NO_2 in a 2-hr exposure which depressed the apparent photosynthetic rate by 7%, whereas 0.25 ppm of SO_2 alone or 0.40 ppm NO_2 alone was required to cause a 2% depression.

Mandl *et al.* (1975) studied effect of SO_2, HF alone and in mixture on barley, sweet corn, and bean plants. A synergistic response was found to a mixture of low

concentrations of two gases. Barley leaves displayed greater injury in a mixed gas experiment than the sum of the injuries caused by the individual pollutant in a 27-day exposure period. The concentrations of the pollutants utilized were approximately 0.08 ppm SO_2 and 0.6 ug/m^3 HF. Interaction of sulphur dioxide with other pollutants on soybean are shown in Table 9.

Interaction of Nematodes with other Soil-borne Plant Pathogens

The number of soil microorganisms present in the root zone of any crop plant provide sufficient opportunity for diverse interactions. Majority of root diseases have a complex etiology (Grogan, 1981; Wallace, 1978). Microorganism in the root zone may exhibit conditions like synergism and antagonism and may interact with each other and with the host. Fawcett (1931) stated that "nature does not work with pure cultures" and that most plant diseases, particularly root diseases, are influenced by associated microorganisms. Root infection by one pathogen may modify the host response to subsequent infection by pathogens or saprohytes.

The term interaction is used quantitatively and qualitatively in describing the interrelationship between two or more factors involved in plant diseases. Synergism and antagonism are best considered as terms describing

TABLE 9

Effect of sulphur dioxide mixture with other pollutants on soybean.

Mixture	Host	Treatment	Effect	Reference
SO_2+O_3	Soybean	0.08 or 0.10 ppm O_3, 0.20 or 0.40 ppm SO_2; 2 or 4 h/day, 1-5 days	Foliar injury and reduced chlorophyll synergistic	Pratt *et al.*, 1983
	Soybean	0.067 ppm O_3, 0.30 ppm SO_2; 7.5 h/day, 5 days	Synergistic reduction in CO_2 exchange rate (CER), no change in CER with O_3 and only slight with SO_2	Le Sueur-Brymer and Ormood, 1984
	Soybean (2 cultivars)	0.25-1.0 ppm O_3, 0.50-1.5 ppm SO_2; 0.75, 1.5 or 3 hours	Foliar injury and reduced shoot fresh weight additive, antagonistic or synergistic, depending upon concentration and time	Heagle and Johnston, 1979
SO_2+NO_2	Soybean	0.2, 0.4, 0.6 ppm of each gases 2; hours	Photosynthesis: synergistic, stomatal conductance: synergistic, respiration: additive	Carlson, 1983
	Soybean (2 cultivars)	0.18 or 0.5 ppm SO_2 alone or with 0.06 ppm NO_2; 4 h/day, 14 days	Yield: no additional effect of NO_2 at low SO_2, but increase in reduction at high SO_2; not designed to determine mixture effects	Amundson, 1983
	Soybean	0.13-0.42 ppm SO_2, 0.06-0.40 ppm NO_2; 3-hours/exposure, 10 days during pod fill (2 year study, ambient air with O_3)	Chlorophyll reduction, synergistic; yield reduced from 9 to 25% in 2 years, synergistic	Irving and Miller, 1984

quantitative plant disease interactions and that the combined effect of a plant parasitic nematode and another plant disease organism in either greater or less than the sum of the effects of the individual organisms.

Nematodes, fungi, bacteria, soil-borne viruses, actinomycetes, etc. are soil inhibiting microorganisms and their pathogenicity to many crop plants are well documented. Plant parasitic nematodes are capable of causing well manifested diseases on their own, but in association with other pathogens they cause much greater damage due to synergistic action of interacting organisms. There are many statements available which suggest that synergistic interactions are common and constantly degrade plant health.

Nematode and fungal plant pathogens

Observations of Atkinson (1892) on the interaction between *Meloidogyne* spp. *Fusarium* wilt of cotton probably tend to researches on disease complexes. He observed that *Fusarium* wilt of cotton was always more severe in the presence of root-knot nematodes. Many investigators have observed that *Fusarium* wilt of cotton caused by *Fusarium oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Synd & Hans. was greatly increased in presence of *Meloidogyne incognita* (Martin *et al.*, 1956; Smith, 1948; 1948; Norton, 1960; White, 1962; Brodie and Cooper, 1964; Maier, 1964; Minton and

Minton, 1966). Porter and Powell (1967) observed that preinoculation with nematodes caused a significant increase in fungal infection. Many investigators have studied the possible interaction of nematodes with other soil inhabiting microorganisms (Powell 1963, 1971a, 1971b; Pitcher, 1965; Bergeson, 1972; Khan, 1984; Webster, 1985; Mai and Abawi 1987; Taylor, 1991).

The mechanism by which *M. incognita* predispose cotton plants to disease has not been fully explained. *M. incognita* may transport the *Fusarium* wilt fungus into roots or the fungus may invade through wounds made when *M. incognita* enters the roots (Smith, 1954). Caquil and Shepherd (1970) concluded that stress and debilitation of seedlings caused by *M. incognita* damage may be major factor in the predisposition, and *M. incognita* induced giant cells may attract and/or be more easily parasitized by fungi than normal cells. Sidhu and Webster (1977) proposed that *M. incognita* may induce production of a translocatable factor or factors that can predispose plant parts distant from the site of *M. incognita* infection. Root splits are caused by *M. incognita* (McClure *et al.* 1973; Shepherd, 1970), and the split may be avenues through which the *Fusarium oxysporum* f. sp. *vasinfectum* and other disease-causing organisms invade roots (Shepherd, 1970).

Some fungi are seed-borne, this indicates that in these cases the fungus is the first active pathogen. Similarly in these cases where fungicidal seed treatments are not effective or when non-fumigant nematicides that have systemic activity are used, fungus become the primary, and nematode the secondary pathogen in sequential interactions. The increase in nematode population detected in a number of studies where the fungus was the primary pathogen demonstrate the importance of this type of interactions (Mountain and Mckeen, 1962; Nordmeyer and Sikora, 1983).

Interaction studies involving nematodes have generally followed the sequential disease complex approach which involves a primary pathogen, the nematode, and a secondary pathogen, the fungus or bacterium etc. The use of sequential inoculations could mask or prevent interaction from developing and alter those affected by the rhizosphere physiochemical environment near the root that change drastically with plant age (Taylor, 1979; Webster, 1985). Change in root exudation patterns that develop with plant age could alter the environment and affect interpretation of results. Activities of microorganisms in the rhizosphere and rhizoplane are greatly influenced by the quality and quantity of root exudation and sloughed-off tissues (Cook and Baker, 1983). It has been often suggested that root exudates are altered by infection of host plants by plant-

parasitic nematodes, particularly root-knot nematodes. Van Gundy *et al.* (1977) observed that exudates from *M. incognita* infected tomato roots attracted hyphae of *Rhizoctonia solani*, enhanced initial sclerotial formation; and increased severity of root decay. Van Gundy *et al.* (1977) and Melakebrahan *et al.* (1985) reported that root exudates from root-knot infected roots were found to contain higher concentrations of Ca, Mg, Na, K, Fe and Cu, as compared to those from non-infected roots. Root exudates generally accumulate near the root tip, and thus the soil microflora are also abundant and close to the location of entry for larvae of *M. incognita* and hyprae of fungus. This section of the review will concentrate on interaction studies involving nematodes especially root knot nematodes and wilt causing and root rot fungi that would be relevant to the proposed work.

a. Interaction with wilt causing fungi

The interaction between *Meloidogyne* spp. and wilt causing fusaria have been studied more than any other nematode-fungus combinations (Powell, 1963; Mai and Abawi, 1987). Martin *et al.* (1956) were the first who conducted their experiments in greenhouses or growth chambers using pasturized, sterilized, or fumigated soil to study the interaction between *Fusarium* wilt fungus and *Meloidogyne* sp.. Interaction between *Meloidogyne* spp. and fungi causing *Fusarium* wilt have been studied different investigators on alfalfa (Griffin, 1986; McGuire *et al.*, 1958); banana (Loos,

1959; Newhall, 1958); beans (Riberio and Ferraz, 1984; Singh *et al.*, 1981); Cabbage (Fassuliotis and Rau, 1969); carnation (Schindler *et al.*, 1961); chrysanthemum (Johnson and Littrell, 1969); cotton (Atkinson, 1892; Cauquil and Shepherd, 1970; Cooper and Brodie, 1933; Garber *et al.*, 1979; Martin *et al.*, 1956; Minton and Minton, 1966; Norton, 1960); cowpea (Thomason *et al.*, 1959); mimosa (Gill, 1958); muskmelon (Bergeson, 1975); pea (Davis and Jenkins, 1963); tobacco (Melendez and Powell, 1967; Porter and Powell, 196- and tomato (Abawi and Baker, 1984; Binder and Hutchinson, 1959; Bowman and Bloom, 1966; Fattah and Webster, 1983; Goode and McGuire, 1967; Hirano *et al.*, 1979; Jenkins and Coursen, 1957; Jones *et al.* 1976; Kawamura and Hirano, 1967; 1968; Orion and Hoestra, 1974; pitcher, 1978; Sidhu and Webster, 1974, 1977, 1979, 1983; Young, 1939, 1940).

Much work has been done on the interaction of root infecting fungi with endoparasitic nematodes in comparison to semi-endoparasitic or ectoparasitic nematodes (Khan, 1984). The interaction of nematodes with wilt causing fungi especially *Fusarium* spp. have drawn much attention. (Powell, 1971a, 1971b). McGuire *et al* (1958) studied the effect of root-knot nematodes on the development of *Fusarium* wilt in Buffalo variety of alfalfa. At termination, the percentage of plants infected with *Fusarium* wilt observed by them are as follows: *Meloidogyne hapla* & *Fusarium oxysporum* f. spp.

vasinfectum 95, *M. javanica* & *F. oxysporum* f. sp. *vasinfectum* 60, *M. incognita* & *F. oxysporum* f. sp. *vasinfectum* 60, *M. incognita* & *F. oxysporum* f. sp. *vasinfectum* 50, *M. arenaria* & *F. oxysporum* f. sp. *vasinfectum* 50, *M. incognita acrita* and *F. oxysporum* f. sp. *vasinfectum* 15, *Meloidogyne* spp. alone and check 0, percentage of dead plants in the above treatments was 40, 5, 10, 4, 0, 3, 0 respectively. Kumar *et al.* (1988) observed that inoculation of either of *M. incognita* or *F. oxysporum* f. sp. *ciceris* reduced growth of chick-pea and highest reduction were obtained with simultaneous inoculation of both pathogens followed by the treatment in which the nematode inoculation preceded the fungus by 10 days. Multiplication of nematodes and galling was reduced in the presence of fungus. Mani and Sethi (1987) studied the effect of the combined incoula of *M. incognita*, *Fusarium oxysporum* or *F. solani* on growth of chick-pea which was found to be additive in nature. However, when nematode was established earlier than the two fungi, the resultant effect was more than simple additive. All the three organisms affected the rhizobial nodulation considerably. Occurrence of *M. incognita* in combination with *F. oxysporum* f.sp. *ciceris* and *F. solani*, not only increased the severity of disease but also shortened the incubation period for disease expression. *F. oxysporum* f. sp. *ciceris* did not affect the nematode population significantly. Patel *et al.* (1987) also observed increase in incidence of chick-pea wilt due to *F.*

oxysporum f. sp. *ciceris* in presence of *M. incognita*. Simultaneous inoculation of both pathogens resulted in more wilt percentage. Martin *et al.* (1956) studied interaction of *Fusarium oxysporum* f.sp. *vasinfectum* causing wilt of cotton with five nematodes viz., *M. incognita*, *M. incognita acrita*, *Trichodours* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. Of these nematodes, only *M. incognita* and *M. incognita acrita* significantly increased incidence of wilt in the both wilt susceptible and wilt resistant varieties. The effect of *M. incognita* Race 3 and *F. oxysporum* f. sp. *vasinfectum* on wilt expression of cotton was studied by Ibrahim *et al.* (1982). They observed that presence of nematode enhanced the development and severity of wilt. Expression of wilt symptoms was observed 10 days earlier in the case of infection with both organisms than with the fungus alone and reduction in plant growth were almost more than two-folds when the two pathogens were present together. Palmer and MacDonald (1974) studied maize root-knot complex caused by *Fusarium* spp. in the presence of various plant parasitic nematode species including *M. incognita*, *Pratylenchus penetrans*, *P. herincissus*, *P. scribneri*, *P. nanus* and *Tylenchorhynchus martini*. Average dry root and shoot weight of maize seedling inoculated with *M. incognita* and *F. maniliforme* were less than those of seedling inoculated with either organisms alone. Goel and Gupta (1984) studied interaction between *M. javanica* and *F. oxysporum* f. sp. *cieris*. They found that

shoot length was minimum when the fungus was inoculated a week after nematode inoculation, whereas root length, fresh shoot and root weights were significantly less when fungus were inoculated a week before nematode inoculation. After two years in the same field of study they observed maximum and significant reduction in shoot length and fresh weight when both the pathogens were inoculated simultaneously. A maximum and significant reduction in number of galls was recorded when nematode and fungus were present together. The reduction in the presence of *F. oxysporum* f. sp. *ciceris* was attributed either to poor and delayed nematode development or to inhibitory effect of fungus on hatching of egg masses (Goel and Gupta, 1986). In pot experiments, Upadhyay and Dwivedi (1987) observed that wilt symptoms were greatest in chick-pea plants inoculated simultaneously with both *M. javanica* and *F. oxysporum* f. sp. *ciceris*, followed by severity by inoculations of the nematode preceding the fungus and the fungus preceding the nematode. The maximum number of root galls was recorded on roots inoculated with the nematode alone and the minimum number on roots where inoculation of the fungus preceded that of the nematode. The maximum reduction in shoot weight occurred where inoculation of the nematode preceded that of the fungus.

Padila *et al.*, (1980) observed that *Meloidogyne* spp. alone caused severe stunting, *F. oxysporum* alone caused no detrimental effect, whereas concomitant inoculation of the

nematode and fungus at planting caused the death of the plants after 45 days.

Fungi non-pathogenic to a host plant may become pathogenic in presence of nematodes. *F. oxysporum* f. sp. *lycopersici* was not pathogenic to cucumber plant, but wilt symptoms developed when *M. incognita* was in association of fungus, but *F. oxysporum* f. sp. *cucumerinum* did not produce symptom on tomato plants even when *M. incognita* was present (Pelez *et al.*, 1983). Smits and Noguera (1982) observed that *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici* were pathogenic to only those eggplant seedings that were infected with *M. incognita*. Lopes and Lordello (1979) studied the wilt of *Piper nigrum* and observed that more damage was done, when *M. incognita* and *F. solani* f. sp. *piperi* were present together.

Nogurea and Smits (1982) suggested that root exudates of tomatoes infected with *M. incognita* stimulate soil microflora reproduction and account for the greater number of colonies (per gram soil) of bacteria and fungi in infected than in healthy plant. This increase, lowered the number of actinomycetes antagonistic to *F. oxysporum* f. sp. *lycopersici* and enhanced its pathogenic effect. Nogurea (1980) observed that germination and growth of *F. oxysporum* f. sp. *lycopersici* in root extracts of a *Fusarium* resistant tomato was significantly greater when tomatoes had been infected

with *M. incognita*. After four weeks of nematode infection, the amount of amino acids in the roots had increased by 50% and carbohydrates by 112% and this was assumed to be the reason for the breaking of resistance to *Fusarium*. Influence of *M. incognita* on the localization of *F. oxysporum* f. sp. *lycopersici* in tomato was studied by Noguera (1983). He observed that fungal penetration was not restricted to nematode site but occurred throughout the root cortex and advanced into the stem via the xylem. Nematode attack appeared to cause systemic change in the plant tissue. Montalro and Melendez (1986) studied histopathology of interaction between *F. oxysporum* f. sp. *dioscoreae* and two nematode species on yam. Examination of root tissues showed that fungal colonization occurred indiscriminately, either inter-or intracellularly. Colonization in the stem was restricted to the vascular system, with abundant proliferation of hyphae, mainly in the nodal tissues. Apparently *M. incognita* and the fungus colonized in different sectors of the invaded tissues, though abundant and vigour hyphae were present in tissues modified by *M. incognita*, indicating a positive interaction. *Pratylenchus coffeae* was sometimes observed with the fungus within the same tissues, but this association had no effect on severity or incidence of wilt. Kleineke-Borchers and Wyss (1982) observed that mixed infection of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato plants resulted in an increase in

indole-acetic acid which may be responsible for quicker appearance of typical wilt symptoms. Nogurea (1982) observed that purified extracts of tomato roots infected with *M. incognita* did not show rishitin, an anti-fungal substance normally present in healthy plants. Nematode free plants also showed the formation of several well developed tyose in the xylem vessels of stem whereas in infected plants these were absent or very reduced. They suggested that the loss of resistance of this tomato variety to *F. oxysporum* f. sp. *lycopersici* infection was associated with the nematode infection which interfered with rishitin and tyose production. Morrell and Bloom (1981) studied effect of temperature on expression of wilt symptom on tomato plants inoculated with *M. incognita* and *F. oxysporum* f. sp. *lycopersici* and observed that wilt severity was increased at 21^{o}C but not at 16^{o}C. Hillocks (1986) studied localised effect of nematode on initial infection and a systemic effect on subsequent disease development of plants. To achieve this, cotton seedlings were grown with a divided root system and inoculated with root-knot nematode, *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* on the same or opposite halves of the root system. Wilt indices were increased by the nematode, but only in plants where both organisms were together on the same parts of the root system. However, in a second experiments in which plants growing in *Meloidogyne* infected soil were stem inoculated with the wilt pathogen,

the nematode caused an increase in wilt severity, despite the physical separation of the two organisms.

Root-knot nematodes are found associated with other wilt causing fungi also. Shoemaker and Barker (1979) in a microplot experiment observed that *M. incognita* had no significant effect on *Verticillium dahaliae* wilt of tomato. Goswami *et al.* (1970) reported high degree of synergism between *M. incognita* and *Sclerotium rolfsii* in brinjal when inoculated together. Prasad *et al.* (1980) studied the association of *Corticium rolfsii* and *M. incognita* in wilt complex of *Solanum khasianum*, a medicinal plant. They found that 42% of the plants wilted, where the fungus and the nematodes were combined, but 12.5% wilted with the fungus alone and no wilt occurred with the nematode alone. Venkata Rao *et al.* (1973) reported an association of *M. incognita* var. *acrita* on *Phytophthora parasitica* var. *nicotianae* on piper-betel roots. Azam *et al.* (1977) observed that presence of *M. incognita* considerably increases the incidence of disease caused by *Rhizoctonia solani*, *Pythium* sp. and *Colletotrichum atrametarium*, on eggplant.

Synergism and antagonism have been observed between fungi and other endoparasitic nematodes than root-knot nematodes. Jorgenson (1970) observed that interaction between *Heterodera schachtii* and *Fusarium oxysporum* was disadvantageous to the nematode and damage to sugerbeet

plants were less when the fungus and nematodes were present than when only the nematode was present. Edward and Singh (1979), while studying the interaction between *Heterodera cajani* and *F. udum* on pigeon-pea, found that the nematode alone caused less damage to var. Type-21 than when associated with *F. udum*. Hasan (1984) observed synergism between *H. cajani* and *F. udum* attacking *Cajanus cajan*. He found a significant increase in wilting when *H. cajani* and *F. udum* were associated with pigeon-pea. Seinhorst and Kuniyasu (1971) reported that rate of multiplication of *Rotylenchulus reniformis* on pea var. "Rando" was increased by the presence of *F. oxysporum* f. *pisi* race-1. Krishnaprasad and Padanganur (1980) observed that soil from *Verticillium* wilt affected plant harboured more *Rotylenchulus reniformis* population compared to that of healthy plants. They concluded that *R. reniformis* might be associated with *Verticillium* wilt of cotton as evidenced by fairly high population of nematodes in the rhizosphere and roots of diseased plants. Mountain and Mckeen (1962), while studying the interaction of lesion nematode, *Pratylenchus penetrans* with *Verticillium dahliae* on tomato, observed that reproduction of nematode was enhanced in such an interaction. In *Verticillum*-wilt of potato, the damage were more when lesion nematode was present (Morsink and Rich, 1968). Burpee and Bloom (1974) observed that incubation period of *V. albo-atrum* was decreased in the presence of *P. penetrans*. In another report, they showed that

Pratylenchus penetrans in roots or rhizosphere were suppressed in the presence of *Verticillium* (Burpee and Bloom, 1978). Ferraz and Lear (1976) found a syngergistic interaction between *F. oxysporum* f. sp. *dianthi* and nematodes such as *Criconemoides curvatum*, *Pratylenchus dianthus* and *M. hapla* on carnation. Grujicic *et al.* (1984) examined the lucerne field and reported that damage due to wilt and rot was greater when *P. penetrans* and *F. oxysporum* var. *medicaginis* were present together. Riedel *et al.* (1985) reported a differential interactions of *Pratylenchus crenatus*, *P. penetrans* and *P. scribneri* with *V. dahliae* in potato early dying disease. Sting nematode, *Belonolaimus longicaudatus* have been found to influence incidence of cotton wilt caused by *F. oxysporum* f. sp. *vasinfectum* (Cooper and Brodie, 1963).

Root-knot nematodes have been found to break the resistance of the crop plants against soil-borne pathogens. Carter (1978) reported enhancement of wilt severity in the completely (polygenic) resistant cultivars of tomato as a result of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* interaction. Hadiastono (1981) observed that tomato varieties originally resistant to *F. oxysporum* became susceptible when inoculated with *Meloidogyne* spp. Harrison and Young (1941) observed that root-knot nematodes reduced the wilt resistance of many varieties of tomatoes. In a glasshouse experiment 20

tomato lines and varieties inoculated with a *Fusarium* plus *Meloidogyne* were found to be susceptible including those resistant to *F. oxysporum* f. sp. *lycopersici*. Donald and Johnson (1973) inoculated eight cultivars of watermelon with known reactions to *Fusarium* wilt with *M. incognita* and observed that wilt symptoms were increased more in resistant than in susceptible cultivars. Johnson and Littrell (1969) observed that *M. incognita*, *M. hapla* and *M. javanica* did not break resistance of chrysanthemum against *F. oxysporum*. O'Ereole *et al.* (1982) in an investigation involving four varieties of tomato showing resistance to *M. incognita* and one not resistance showed that infection with *F. oxysporum* f. sp. *lycopersici* was greater in the nematode susceptible variety than in other more resistant varieties. They observed similar (although less conclusive) results with *Verticillium dahliae*. Kleineke-Borchers and Wyss (1982) noted that prior infection of *M. incognita* followed by *Fusarium oxysporum* inoculation greatly enhanced fungal disease in both *Fusarium* susceptible and resistant tomato plants. Pitcher (1974) also proved that infection of "Pearson VF" tomato which is moderately resistant to *F. oxysporum* f. sp. *lycopersici* became susceptible to this fungus in presence of *M. javanica*. Thomason (1958) found that wilt in *Vigna sinensis* was more severe in plants grown in soil infested with both *F. oxysporum* f. sp. *tracheifilum* and *M. javanica* than in soil infested with fungus alone. McGuire *et al.* (1958) observed

that only 10% plants showed the wilt symptoms when infected with *M. incognita acrita* and 15% with *F. oxysporum* f. sp. *vasinfectum* alone, whereas 95%, 60% and 50% plants became wilted when the fungus was present in combination with *M. hapla*, *M. javanica* and *M. incognita* respectively.

Davis and Jenkins (1963) observed a synergistic response between *Meloidogyne* spp. and *Fusarium*-wilt resistant pea variety. An increase in severity of *Fusarium*-wilt of tomato and increased colonization of fungus in the vascular tissue were observed when *M. incognita* was present (Carter *et al.*, 1977). Garber *et al.*, (1979) found that in soil infested with *F. oxysporum* f. sp. *vasinfectum* and *M. incognita*, the extent of fungal invasion and colonization of cotton was correlated with the nematode galling. Noguera (1983) observed that roots of tomato plants only showed colonization by *F. oxysporum* when infected with *M. incognita*.

b. Interaction with root-rot fungi

Many investigators have noticed that nematodes interact with root-rot fungi synergistically, hence greater damage to plant. Tu and Cheng (1971) found that severity of root-root in kenaf (*Hibiscus cannabinus* L.) was increased when seedlings were infected simultaneously by *Macrophomina phaseolina* and *M. javanica*. Mishra *et al.* (1988) observed that *M. incognita* provided a good site for easy entry of the developing hyphae of *R. bataticola* (*M. phaseolina*) in white

jute (*Corchorus capsularis*) and thus increased the severity of the disease. The number of dead plants were increased significantly when pathogens were inoculated together. Goel and Gupta (1986) observed that irrespective of time, combined inoculation of *M. javanica* and *R. bataticola* on chick-pea reduced growth parameters compared with when inoculated alone. The number of root galls was significantly reduced when nematode was inoculated 7 days before the fungus. Bruton and Heald (1987) studied effect of *M. phaseolina* on cantaloupe in the presence of *M. incognita* and found reduction in top dry weight of the plant. Apt and Koike (1962) studied the relationship of *M. incognita acrita* with *Pythium graminicola* on sugarcane. Colin and Powell (1971) reported an extensive fungal colonization in the root-rot susceptible cultivar of tomato "Dixie Bright 101" when *M. incognita* preceded *R. solani* by 21 days. Sharma *et al.* (1980) reported a syngergistic effect on the root-rotting of okra plants when *M. incognita* and *R. bataticola* were combined together. Al-Hazmi (1985) reported that severity of root-rot caused by *M. phaseolina* in root-rot disease complex of French bean (*Phaseolus vulgaris*) increased when the nematode was introduced 2 weeks before the fungus, and if the fungus was introduced first, then the nematode infection and reproduction were reduced. Chhabra and Sharma (1981) in a pot trial with okra and eggplant observed that *M. incognita* and *R. bataticola* significantly reduced the germination of both

TABLE 10

Interaction of root-knot nematodes with wilt causing Fusaria and root-rot fungi.

Meloidogyne spp.	Associated organism	Host	Comments	References
M. incognita	*Fusarium equiseti*	Pea	Preponded wilt symptom appearance	Chahal and Chhabra, 1985
M. incognita	*F. oxysporum* f. sp. *nicotianae*	Tobacco	Significant infection occurred only when both pathogens were present	Porter and Powell, 1967
M. incognita	*F. oxysporum* f. sp. *ciceris*	Chickpea	Increased wilt severity and shortened incubation period of disease expression	Mani and Sethi,1987; Patel *et al.*, 1987; Kumar *et al.*, 1989
M. incognita	*F. solani*	Chickpea	Increased wilt severity and shortened incubation period of disease expression	Mani & Sethi, 1987
M. incognita	*F. oxysporum* f.sp. *melonis* *F. oxysporum* f.sp. *lycopersici*	Bringal	Non-pathogenic fungi caused severe vascular necrosis of the shoots	Smits & Noguera, 1982
M. incognita	*Fusarium* spp.	Watermelon	Increased wilt severity	Donald & Johnson, 1973
M. incognita	*Fusarium moniliforme*	Maize	Increased root-rot severity	Palmer & MacDonald, 1974
M. incognita	*F. oxysporum* f.sp. *lycopersici*	Cucumber	Non-pathogenic fungus caused wilting in presence of nematode	Pelez *et al.*, 1983
M. incognita	*F. oxysporum* f.sp. *lycopersici*	Tomato	Increased wilt severity	Morrell and Bloom, 1981; Kleineke-Borehers and Wyss, 1982; Carter *et al.*, 1977.

Table 10 contd..

Meloidogyne spp.	Associated organism	Host	Comments	References
M. incognita	*F. oxysporum* f.sp. *vasinfectum*	Cotton	Increased wilt severity	Cooper & Brodie, 1963; Martin *et al.*, 1956; Ibrahim *et al.*, 1982; Hillocks, 1986
M. incognita	*F. oxysporum* f.sp. *pisi*	Pea	Increased wilt severity	Padilla *et al.*, 1980
M. incognita	*F. oxysporum* f.sp. *vasinfectum*	Alfalfa	Increased wilt severity	McGuire *et al.*, 1958
M. incognita	*R. bataticola*	French bean	Increased severity of root-rot	Al-Hazmi, 1985
M. incognita	*R. solani*	Cotton	Synergism occurred	Carter, 1975
M. incognita	*R. solani*	Tobacco	Worst infection occurred when nematode inoculated 10 or 21 days before fungi	Batten and Powell, 1971
M. incognita	*R. solani*	Potato	Increased disease severity	Sharma and Gill, 1979
M. incognita	*R. solani*	Okra	Simultaneous inoculation caused Max reduction	Chhabra *et al.*,1977
M. incognita	*M. phaseolina*	White jute	Increased disease severity	Mishra *et al.*, 1988
M. incognita	*R. solani*	Tomato	Increased fungal colonization	Colin and Powell, 1971
M. incognita	*R. solani*	French bean	Increased disease severity	Reddy *et al.*,1979
M. incognita	*R. bataticola*	Okra	Increased root-rot	Sharma *et al.*, 1980
M. incognita acrita	*F. oxysporum* f.sp. *pisi*	Pea	Synergistic response observed, broke resistance	Davis and Jenkins, 1963

Table 10 contd..

Meloidogyne spp.	Associated organism	Host	Comments	References
M. incognita acrita	*R. solani*	Cotton	Disease increased	Reynolds and Hanson, 1957
M. incognita acrita	*F. oxysporum* f.sp. *cubense*	Banana	Incubation period of fungus shortened	Loos, 1959
M. incognita	*R. solani*	Tomato	Reduction in growth parameters	Chahal and Chhabra, 1984
M. javanica	*F. oxysporum* f.sp. *ciceris*	Chickpea	Significant reduction in growth parameters	Upadhyay and Kusum Dwivedi, 1987; Chhabra *et al.*,1977; Goel and Gupta. 1984 1986
M. javanica	*F. oxysporum* f.sp. *tracheiphilum*	Cowpea	Increased wilt severity	Thomason *et al.*,1959
M. javanica	*F. oxysporum* f.sp. *melonis*	Muskmelon	Increased wilt severity, broke resistance	Orion and Netzer, 1981
M. javanica	*F. oxysporum* f.sp. *lycopersici*	Tomato	Susceptibility to wilt increased	Jenkins and Coursen, 1957; Bergeson *et al.*, 1970
M. javanica	*F. oxysporum*	Chrysanthemum	Early and severe wilt appearance	Johnson and Littrell, 1969
M. javanica	*F. udum*	Pigeonpea	Susceptibility to wilt increased	Abdul Salam and Khan, 1986
M. javanica	*R. bataticola*	Chickpea	Reduced growth parameters	Goel and Gupta, 1986
M. javanica	*R. bataticola*	Tomato	Increased wilting incidence	Goswami *et al.*, 1976
M. javanica	*R. solani*	Cowpea	Plant growth reduced when nematode inoculation was done 3 weeks prior to the fungus	Kumar *et al.*, 1988

Table 10 contd..

Meloidogyne spp.	Associated organism	Host	Comments	References
M. javanica	*M. phaseolina*	Kenaf	Increased severity of root-rot	Tu and Cheng, 1971
M. javanica	*R. bataticola*	French bean	Reduced growth parameters	Reddy *et al.*, 1979
M. hapla	*F. oxysporum* f.sp. *lycopersici*	Tomato	Wilt incidence increased	Jenkins and Coursen, 1957
M. hapla	*F. oxysporum* f.sp. *vasinfectum*	Alfalfa	Severe wilting appeared	McGuire *et al.*,1958
M. hapla	*F. oxysporum*	Chrysanthemum	Wilt incidence increased	Johnson and Littrell 1969
M. arenaria	*F. oxysporum* f.sp. *nicotianae*	Tobacco	Significant infection occurred only when both pathogens present	Porter and Powell, 1967
M. arenaria	*F. solani*	Ground nut	Earlier appearance of wilt symptoms	Patel *et al.*, 1985
Meloidogyne spp.	*F. oxysporum* f.sp. *lycopersici*	Tomato	Resistance broken	Almedia, 1972
Meloidogyne spp.	*F. oxysporum*	Tomato	Resistance broken	Hadiastono, 1981

species when they were present together in the substrate. Goswami *et al.* (1976) noted that when *M. javanica* was inoculated onto tomato plants, 3-weeks before infection with *R. bataticola*, 33.3% wilting occurred. Approximately 13.3, 6.7 and 6.7% willey was noted after simultaneous pathogen inoculation; fungus infection alone or fungus infection 3-weeks before nematode inoculations, respectively. Sakhuja and Sethi (1986) observed that multiplication of *M. javanica* and the galling on groundnut was adversely affected by *bataticola* and *F. solani*. Reduction in multiplication was maximum wherever one or both the fungi were inoculated simultaneously with the nematode, but *R. bataticola* proved to be more antagonistic to *M. javanica*.

Khan and Muller (1982) studied the interaction between *R. solani* and *M. hapla* on radish (*Raphanus sativus*) in gnotobiotic culture in petridishes. This experiment indicated that the physiological changes in the galled region due to nematode infection predisposed the roots for invasion and rapid colonization by the fungus. Reddy *et al.* (1979) reported that root-knot nematode, *M. incognita* and *R. solani* are often found associated simultaneously with roots of french bean (*Phaseolus vulgaris*) under field conditions. Reduction in growth parameters were observed when both pathogens were present at a time. Kanwar *et al.* (1988) observed that growth of cowpea was significantly reduced

when *M. javanica* was inoculated 3 weeks prior to *R. solani*. The number of bacterial nodules and galls was reduced significantly in presence of both pathogen with maximum reduction in nematode alone treatment. Sharma and Gill (1979) observed significant reduction in shoot weight of potato plant inoculated with *M. incognita* and / or *R. solani* and maximum reduction in multiplication of nematode (66.72%) occurred when *R. solani* was inoculated before *M. incognita*. Chahal and Chhabra (1984a) reported that *M. incognita* and *R. solani* separately as well as in combination significantly reduced shoot length, shoot and root weights of tomato. They further observed that inoculation of tomato seedlings with *M. incognita* and *R. solani* reduced seed germination to 18% compared with 48% and 39% in the case of single inoculation of *M. incognita* and *R. solani* respectively (Chahal and Chhabra 1984b). Chhabra *et al.* (1977) showed that interaction involving *R. solani* and *M. incognita* on okra significantly reduced shoot and root lengths and wet and dry weights. Hazarika and Roy (1975) examined the interrelationship between *R. solani* and *M. incognita* on eggplant and found that number of galls and egg masses on roots were significantly greater in plants inoculated with nematode and fungus together than in those inoculated with nematode alone. Chhabra et al (1977) observed that maximum reduction in growth parameters of okra was obtained when *M. incognita* and *R. solani* were inoculated simultaneously.

Fungus with mechanical injury of roots was more damaging to plants than the fungus alone. Chhabra *et al.* (1978) studied the influence of soil types on the interaction of *R. solani* and *M. incognita* and found that inoculation of okra with both the pathogens markedly reduced shoot and root growth. Nematode population were much denser in sandy loam as compare to loamy sand or sandy clay loam, but number of galls did not vary between soil types.

Powell *et al.* (1971) noticed that soil-borne fungi like *Pythium*, *Curvularia* and *Botrytis* caused extensive root decay when root-knot nematode preceded the fungi. *M. incognita* was found associated in a root-rot with *F. concolar* in papaw and with *F. oxysporum* in tomato (Saeed *et al*, 1972). *M. arenaria* appeared to predispose the maize plant to *S. rolfsii* (Minton *et al.*, 1975). Welty *et al.* (1980) found that *Phytophthora megasperma* f. sp. *medicaginis* intensified root-rot severity in resistant and susceptible lucerne when *M. hapla* or *M. incognita* were present. Mukhtar and Khan (1989) observed greatest suppression in chick-pea plant when they were inoculated simultaneously with *M. javanica* and *S. (Corticium) rolfsii*, followed by the fungus prior to nematode.

Besides root-knot, other nematodes also have been found to interact with root-rot fungi. Kisiel *et al.* (1969) studied the possible interactions between nematodes, *Tylenchus agricola* and *Tylenchorhynchus claytoni* on root-rot

of corn caused by *F. roseum* and *pythium ultimum*. Nematode population increased to a greater degree in plants incoulated with *F. roseum* than with *P. ultimum*. Penetration of stele of *F. roseum* was significantly greater in presence of nematodes. They concluded that nematode may in some cases increase the severity of the disease, but the fungus was by so far the dominant pathogen in this relationship. Littrell and Johnson (1969) observed an interaction in combined inoculation with *Pythium aphanidermatum* and *Belonolaimus longicaudatus* on chrysanthemum. Sobun *et al.* (1979) reported an interrelationship between *Tylenchorhynchus* and root-rot fungus on chick-pea. Upadhyay and Swarup (1981) observed a significant reduction in growth of maize plant, when both *Tylenchorhynchus vulgaris* and *F. moniliforme* were inoculated together. Abawi *et al.* (1985) studied the disease complex of *Phaseolus vulgaris* caused by one or more species of *Fusarium*, *Rhizoctonia*, and *Thielaviopsis* with lesion nematode, *Pratylenchus* spp. Studies on interactions of *Verticillium dahliae, Colletotrichum coccodes, Rhizoctonia solani* and *Pratylenchus penetrans* in the early dying syndrome of potato revealed an additive symptom expression, when *P. penetrans* combined with *V. dahliae* (Kotcon *et al.*, 1985).

Interaction of nematode and fungal plant pathogens under pollutant stress

Apparently no attempt has been made to determine the impact of air pollution stress on fungus-nematode interaction

in disease complexes. Effects of some heavy metals as soil pollutants have been studied by some workers in nematode-fungus complexes. Khan and Abul Salam (1990) studied effects of nickel (Ni) and cobalt (Co) at different concentrations on interaction of *Meloidogyne javanica*, *Fusarium udum* and *Rhizobium* on pigeonpea. They observed that *M. javanica* and *F. udum* interacted synergistically and caused more severe wilting symptoms than *F. udum*, but the result was reverse in the presence of Ni, i.e. wilting was completely inhibited, root galling was suppressed but it caused reduction in plant growth and nodulation. They suggested that this may be due to toxic effect of nickel towards the pathogens. It was found that cobalt too, suppressed the wilting symptoms on the test plants by *F. udum*, and had similar effect as a nickel. Cobalt increased galling produced by *M. javanica* and caused a significant reduction in growth parameters of the plant.

The review of literature shows that in recent years some studies have been conducted on interactions between biotic pathogens (including fungi and nematodes) and some gaseous air pollutants. The ability of some biotic pathogens, particularly viruses, bacteria and fungi to influence the susceptibility of the plants to air pollutants has been Whether the nematodes also influence the plant susceptibility to air pollutants is yet to be explored fully. The effect of gaseous air pollutants on the interactions between nematodes

and fungi has not gained study. Whether the disease intensity caused by nematodes or fungi or the disease severity developing through their interactive effect in a synergistic relationship are influenced adversely or favourably or whether this relationship is impaired on air pollution stressed needs to be examined. The present study is planned to answer some of these questions.

MATERIAL AND METHODS

The method to be employed and steps to be taken for obtaining the materials for conducting the proposed investigations will be as follows:

1. Root-knot nematode population

a. Collection of field populations:

Field plots grown with vegetables and pulses in and around Aligarh will be surveyed to collect root samples infected with root-knot nematodes. Root samples collected in polythene bags and properly marked will be brought to the laboratory for species identification and establishing of single egg mass population.

b. Identification of species

The species of root-knot nematodes present in the root samples will be identified by using perineal pattern characteristics of mature females (Eisenback *et al.*, 1981) and by conducting North Carolina host differentiate tests (Taylor and Sasser, 1978).

(i) Perineal pattern method

For the identification of species of *Meloidogyne* present in root samples, mature females will be dissected out from the galls of the root samples. Perineal patterns of females (10-20) from each sample will be prepared and examined under microscope to study their characteristics. The

species will be identified on the basis of perineal pattern characteristics (Eisenback *et al.*, 1981).

(ii) North Carolina host differential test:

The species of *Meloidogyne* present in the root samples will be maintained on tomato (cv. Pusa Ruby) or eggplant (cv. Pusa Kranti) in pots. Chopped roots from the samples will be added to pots filled with steamed sterilized field soil. Tomato or egg plant seedlings will be planted in the pots. From these field populations, single egg mass cultures of the species of *Meloidogyne* will be raised on tomato or eggplant grown in steam-sterilized soils in pots. For conducting North Carolina host differential tests to establish the identity of species, seedings of tomato cv. Rutgers, tobacco, cv. NC95, pepper cv. California Wounder, peanut cv. Florrunner, watermelon cv. Charlston Gery and cotton cv. Delptapine 61 will be grown in clay pots containing sterlized soils in pots in triplicate. Two additional pots of tomato will be included to determine the time of termination of test. Plants will be inoculated with 5,000 freshly hatched second stage juveniles (J2) per pots in the vicinity of roots of test plants and pots will be kept at glasshouse benches (temp. 27-30^{o}C). The roots will be harvested 50-60 days after inoculation and will be washed thoroughly with tap water and examined for the presence of galls and egg masses. In case of very light infection, roots

will be stained with phloxine B to visiulize the minute egg masses. Galls and egg masses will be counted and GI and EMI will be rated on 0-5 scale (Taylor and Sasser, 1978).

After rating the root system, result will be compared with the differential host test reaction chart (Table 11) to identify four species of *Meloidogyne* viz., *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. The results will also differentiats races in *M. incognita* and *M. arenaria*. Results will be compared with identification made earlier on the basis of perineal pattern for confirmation of their identity.

c. Maintenance of inoculum

After identification of the species, single egg mass cultures of the species of root-knot nematodes will be raised on tomato or eggplant seedlings in greenhouse conditions by inoculating 10-15 egg masses of single egg mass cultures used in North Carolina host differential tests. Sub-culturing will be done by inoculating new seedlings of tomato or eggplant with at least 15 egg masses, each obtained from the single egg mass culture in order to maintain sufficient inoculum for further studies. The results of the host differential tests will also differentiate races in *M. incognita* populations.

d. Inoculation

The plants in the proposed study will be inoculated in the form of second stage juveniles (J_2). Second stage juveniles will be obtained by incubating egg masses from the

cultures of nematode in sterilized water in petri dishes at 25°C. The number of juveniles (J_2) in the suspension will be standardized before inoculation of the seedlings in pots. The described amount of suspension will be taken by micropippet controller and added near the roots into 4-5 holes made around the seedlings. The holes will be covered with the soil after inoculation. The inoculum level (Pi) of 0,10,100, 1000,10000 and 2000 J_2 per pot is planned to be used.

2. Inocula of *Fusarium oxysporum* f. sp. *ciceris* and *Rhizoctonia bataticola*

Pure cultures of the two fungi, *F. oxysporum* f. sp. *ciceris* (Padwick) Chattopadhyay and Sen Gupta pathogenic on causing wilt on chickpea and *R. batiticola* (Taub.) Butl. causing root-rot on soybean will be obtained from the Division of Mycology or Plant Pathology, Indian Agricultural Research Institute, New Delhi.

a. Maintenance and pure culturing

The fungi will be sub-cultured and maintained on PDA slants as stock cultures. The fungi will be sub-cultured on fresh PDA slants at regular intervals.

b. Prepration of inoculum

In order to obtain enough inocula of the fungi for the experiments, Czapek's liquid medium with the following constituents will be used as a culture medium:

TABLE 11

North Carolina differential host test reaction chart

Meloidogyne species and races	Cotton cv. Deltapine 16	Tobacco cv. NC 95	Pepper cv. California Wonder	Watermelon cv. Charleston Grey	Peanut cv. Florrunner	Tomato cv. Rutgers
M. incognita						
Race 1	[-]	[-]	+	+	-	-
Race 2	[-]	[+]	+	+	-	-
Race 3	[+]	[-]	+	+	-	-
Race 4	[+]	[+]	+	+	-	-
M. javanica	-	+	[-] (+)	+	- (+)	-
M. arenaria						
Race 1	-	+	+	+	[+]	-
Race 2	-	+	+	+	[-]	-
M. hapla	-	+	+	[-]	[+]	-

Box indicates key differential host plant
Parenthesis indicate that a small proportion of the population attacks the host
+ = Susceptible; - = Resistant.

KNO_3	2g
$KH_2\ PO_4$	1 g
$Mg\ SO_4$	0.5 g
Kcl	0.5 g
Sucrose	30 g
Distilled water	1000 ml.

The prepared medium will be filtered through muslin cloth. One hundred ml. of the medium will be transfered into 250 ml. flasks and the flasks will be properly plugged with non-absorbent cotton. Sterilization of the flasks containing the medium will be done in an autoclave at 15 lbs. presssure for 15 minutes. With the help of sterilized inoculation needle, the fungal mycelium will be transfered from the PDA slants to the flaks at laminar flow bench. Inoculated flasks will be incubated at 23-25^{o}C for 7 days to allow mycelial growth of the fungi. At the end of the inoculation period, the content of the flaks will be poured over in a funnel lined with Whatman filter paper No.1. The mycelium mat collected in the funnel will be washed with distilled water to remove the traces of the medium and mycelial mat will be gently pressed between the folds of sterile blotting paper to remove the excess amount of water. Inoculum in form of suspension will be prepared by blending 10 g fungal mycelium in 100 ml. of sterilized distilled water for 30 seconds in a

Waring blender. Thus, each 10 ml. of this suspension will contain about 1 g of the fungus.

3. **Pollutant (Sulphur dioxide):**

a. **Exposure chamber**

Experimental plants grown in pots for the treatments receiving SO_2 exposure intermittently will be exposed to sulphur dioxide in air pollutant exposure chamber (Standard Appliances, Varansi) which is equipped with a full size door, and an exhaust duct at its top to carry out the [illegible] mixture. The chamber's bottom is double walled, upper side has opening, and a blower is attached to the lower side. Voltage supply to blower will be regulated by a voltage controller which is displayed on a panel meter. The potted plants which are to be treated will be kept in the exposure chamber and will be exposed for a desirable length of time. The concentration of the gas, though regulated by the voltage supply, will be determined by sampling of the air in chamber and analysis of the sampled air.

b. **Generation of SO_2 Gas**

By the action of sulphuric acid ($H_2 SO_4$) on sodium sulphite ($Na_2 SO_3$), SO_2 gas will be produced in a generator under contol reaction conditions. The amount of $Na_2 SO_3$ and H_2SO_4 discharged from the reagent bottles mounted over the SO_2 generator will be determined by collecting the solution dropping through capillary tube in a graduated cylinder for

sometime and expressing the rate in ml/min on the basis of flow rate or solution feeding rate. Solution of soidum sulphite ($Na_2 SO_3$) and sulphuric acid (10%) will be prepared, to produce required amount of SO_2 gas/min. On complete reaction 1M Na_2SO_3 produces 1M. SO_2 or 126 mg Na_2SO_3 produces 64 mg SO_2. 10% $H_2 SO_4$ solution will be used for all the working solutions of Na_2SO_3. The reaction is shown below:

$$Na_2 SO_3 + H_2SO_4 \text{ ----------> } SO_2 + Na_2 SO_4 + H_2O$$

c. Exposure and doses

Exposure of the plants will be done intermittently. Seedlings will be exposed to SO_2 when they are 3-4 weeks old at alternate days for 3 h. for each exposure. This procedure will be continued for 75 days. The concentrations of the SO_2 proposed to be used for the exposure will be 0.1 and 0.2 ppm.

d. Air pollution symptoms

The experimental plants of chickpea and soybean treated with SO_2 will be regularly examined for SO_2 induced symptoms. The symptoms if present will be noted and characterized.

e. Sampling

Sampling of the air within the exposure chambers will be done by Handy Air Sampler (Kimoto, Japan) at the rate of 200 ml. air per minute for 3 min. For sampling of gaseous air pollutants, methods being followed at the National

Environmental Engineering Research Institute (NEERI), Nagpur, (Anon, 1986) will be employed.

f. Analysis of sulphur dioxide

Analysis of sulphur dioxide in sample will be done by Weast and Fack method (being used at NEERI). Some details of the analysis, preparation of chemicals are described stepwise, below:

(i) Absorbing reagent (Sodium tetrachloromercurate)

27.2 g meruric chloride and 11.7 g sodium chloride will be dissolved in 1 lit. double distlled water (D.D.W.). This solution will be stored at room temperature and can be used for two months.

(ii) Sulphamic acid (0.6%)

0.6 g sulphamic acid will be dissolved in D.D.W. to bring the volume to 100 ml.

(iii) Formaldehyde solution (0.2%, to be prepared fresh).

0.5 ml formaldehyde solution (40%) will be diluted in D.D.W. to 100 ml.

(iv) Rosaniline hydrochloride solution (0.2%)

a. Step I solution

0.2 g rasaniline hydrochloride will be dissolved in 1 N hydrochloric acid to bring the volume up to 100 ml. It will be kept in refrigerator over night.

b. Step II solution

4 ml. of step I and 6 ml concentrated HCl will be dissolved in D.D.W. to bring the volume to 100 ml.

(v) Potassium dichromate solution (0.1 N)

Exactly 1.226 g dried potassium dichromate will be dissolved in D.D.W. to 250 ml.

(vi) Sodium thiosulphate (0.1 N)

7.0 g sodium thiosulphate will be dissolved in 250 ml D.D.W. For preservation 2 ml. of chloroform will also be added.

(vii) Iodine solution (0.1 N)

10 g of potassium iodine will be dissolved in 20 ml D.D.W. After adding 3 g crystals of iodine, solution will be stirred. Solution will be kept over-night and dilluted up to 250 ml with D.D.W. Solution will be preserved in amber coloured bottle in dark.

(viii) Potassium iodine solution (100%)

10 g of potassium iodine will be dissolved in 10 ml of D.D.W. This may be prepared at the time of titration.

(ix) Starch solution

A paste of 1.25 g soluble starch will be prepared with D.D.W. and will be poured in 250 ml boiling D.D.W. It will be

boiled for 5 minutes with stirring and allowed to cool on stand. Fresh and clear supernatent will be used.

(x) Metabisulphite solution

0.3 g sodium metabisulphite will be dissolved in 500 ml D.D.W. This solution will contain 320 or 400 ug/ml of SO_2.

g. Standardisation of sodium thiosulphate

50 ml D.D.W. will be poured into a 250 ml conical flask and add to it 10 ml of potassium dichromate (0.1 N) 10 H_2SO_4 (1:1) and 1 ml of potassium iodine solution will be added to it. The flask will be kept in dark for 5 minutes for reaction.

Above solution will be titrated with sodium thiosulphate (vi) till a faint yellow colour is obtained. Then 1 ml of starch solution will be added; blue colour will now appear. The titration will be countinued until faint blue colour disappears. Final colour will be distinct bluish green tinge due to chromous ions. Titration reading will be noted. This will be repeated to get constant reading. Then normality of sodium thiosulphate will be calculated according to the following formula.

$$\text{Normality of thiosulphate} = \frac{\text{Normality of dichromate} \times \text{vol. of dichromate}}{\text{Vol. of thiosulphate required}}$$

h. Standardisation of metabisulphite solution

25 ml of iodine solution (vii) will be taken in two 250 ml conical flasks (A & B). In A (blank) and B, 25 ml D.D.W. and 25 ml metabisulphite solution (X) will be added. After keeping flasks in dark for 5 min for reaction, titration will be done with sodium thiosulphate till color become faint yellow. Two ml starch solution will be added and titration will be continued untill blue colour disappears which was produced due to addition of starch. Titration will be repeated to get constant readings and normality of metabibihlphite will be calculated according to the following formula:

$$\text{Normality of metabisulphite solution} = \frac{(A-B) \times N}{Vm}$$

A = volume of sodium thiosulphate required for blank (flask A)

B = Volume of sodium thiosulphate required for metabisulphite (flask B).

N = Normality of thiosulphate calculated earlier

Vm = Volume of metabiosulphite solution taken.

i. Working standard metabisulphite solution in absorbing media

Now solution of metabisulphate will be prepared of such a strength that 1 ml of solution contains 10 ug of SO_2. Totaly ug of SO_2 in metabisulphite solution will be calculated according to following formula:

Y = Normality of metabisulphite x 32000

where Y is total ug of SO_2 in metabisulphite solution:

Now working standard will be prepared in 100 ml abosring media such 1 ml = 10 ug SO_2

$$Z = \frac{10 \times 100}{Y}$$

Z is the volume of metabisulphite required for dilution to 100 ml with absorbing media.

j. Calibration of standard curve

Working standard metabisulphite will be pipetted in graduated amounts (such as 0.1, 0.2, 0.3, 0.5, 0.7, 0.9, 1.0, 1.1, 1.2, 1.5 ml which will contain 1,2,3,5,7,9,10,11,12 and 15 ug SO_2 respectively) into a series of impingers or grauated nessler tubes. Absorbing media will be added to tubes to make volume 10 ml. In the blanks 10 ml of absorbing media will be added only. Then 1 ml sulphamic acid (ii), 2.0 ml formaldehyde solution (iii), 5.0 ml rosaniline hydrochloric solution (iv) will be added one by one, and after adding each, the solution will be shaken gently. After it, the volume of each solution will be maintained with D.D.W. up to 25 ml. After 30 minutes but befor 60 min., the transmittance will be determined at 550 nm in spectrophotometer. A standard curve will be drawn between transmittance and concentration of ug SO_2.

k. Estimation

10 ml of sampled absorbing medium will be taken in impinger. The 1 ml sulphemic acid, 2 ml formaldehyde solution and 5 ml rosaniline will be added one by one and after adding each, the solution will be shaken gently. After 30 min but before 60 min transmittance will be determined at 550 nm in specterophotometer. In the control (blank) in place of absorbing media non-sampled medium will be taken. The ug of SO_2 will be determined by placing of transmittance (%) on calibrated standard curve, the corresponding value (ug SO_2) will be found out. SO_2 ug/m^2 will be calculated according to following formula:

$$SO_2\ ug/m^3 = \frac{ug\ SO_2}{\text{Volume of air sampled (lit)}} \times 10^3$$

Volume of air sampled = Flow rate of air x time of sampling (minutes)

ug of SO_2/m^3 will be converted into ppm according to the following formula:

$$ppm = \frac{ug\ /m^3 \times 22400}{M \times 10^6}$$

M is the molecular weight of the pollutant.

4. Pathogenicity tests

Pathogenicity of *M. incognita*, *F. oxysporum* f. sp. *ciceris*, and *R. bataticola* (=*Macrophomina phaseolina*) at the inocullum levels of 10,100,1000 and 10000 second stage

juveniles (J_2) and 0.5, 1.0 and 2.0g of mycelial suspension per pot will be tested on six cultivars of chickpea and soybean. The relative sensitivity of the cultivars of chickpea and soybean to 0.1 and 0.2 ppm of SO_2 will also be tested.

Plants in pots inoculated with the fungi will be kept in greenhouse. Plants will be observed regularly for wilt symptoms appearance and will be noted. The intensity of wilt symptom, and wilting index will be determined after 15-30-45-60 days of inoculation according to the following scale.

0 = No symptom

1 = Light symptoms

2 = Moderate symptoms

3 = Heavy symptoms

4 = Servere symptoms (dead) (Sidhu and Webster, 1978)

The relative amount of root-kot on a root system in each treatment will be determined by scoring the extent of disease on scale ranging a 0-5 scale (Reddy *et al.*, 1979) as given below:

0 = No root-rot

1 = 1-20% root-rot

2 = 21-40% root-rot

3 = 41-60% root-rot

4 = 61-80% root-rot

5 = 81-100% root-rot

The following treatments will be inocluded for this study:

T_1 = Control

T_2 = 0.5 g of mycelial mat per pot

T_3 = 1.0 g of mycelial mat per pot

T_4 = 2.0 g of mycelial mat per pot

Each treatment will be replicated five times. The same treatments will be used for each cultivar.

Pathogenicity of *M. incognita*

Pathogenicity of root-knot nematode, *M. incognita* will be tested using, 0, 10, 100, 1000 and 10000 J_2 (per pot) inoculum levels. Three week-old seedlings of six cultivars of both crops will be inoculated in pots. Inoculated plants will be kept in greenhouse for 50 days. At the time of termination, plants will be uprooted gently and roots will be washed with tap water, with care to avoid loss and injury to the root system. Growth characters like lengths of root and shoot; fresh and dry weights of shoot and root will be recorded. Number of galls and egg masses per root system will be counted. Gall index and egg mass indices will be rated according to Taylor and Sasser's scale of 0-5 (Taylor and Sasser, 1978). After determining GI and EMI, final population of nematode for each cultivar will be determined by determining numbers of eggs, female population in roots and

soil population of the juveniles and males and Rf will be determined by $Rf = \frac{Pf}{Pi}$ (Oastenbrink, 1966). Treatments included for this study will be:

T_1 Control

T_2 = 10 J_2/pot

T_3 = 100 J_2/ pot

T_4 = 1000 J_2/ pot

T_5 = 10000 J_2/pot

The resistant and susceptibility will be allocated to cultivars according to modified Canto-Saenez scheme (Sasser *et al.*, 1984).

Sensitivity to SO_2

Relative sensitivity of all six cultivars of chick-pea and soybean to SO_2 will be be tested at 0.1 and 0.2 ppm SO_2. Three-weeks-old seedlings will be exposed to SO_2 in exposure chambers every alternate days for 3 h. Exposing of plants to SO_2 will be continued for 75 days. At the end of each exposure, plants will be transfered to the green-house. Treatment are:

T_1 = Control

T_2 = 0.1 ppm SO_2

T_3 = 0.2 ppm SO_2

All cualtivars of both crops will be treated and each variety will be replicated five times.

5. Interaction studies

a. Interaction of *M. incognita* wilt and root-rot fungi

Interaction of *M. incognita* on chickpea with *F. oxysporum* f. sp. *ciceris* and on soybean with *R. bataticola* (=*M. phaseolina*) will be examined first. One susceptible and one resistant cultivar to the respective fungus will be selected considering the results of pathogenicity tests. Three-weeks-old seedling of the cultivars of both crops, raised from surface sterilized seeds in 30 cm dia pots will be inoculated with J_2 of the nematode. Fungal inoculation will be done in the form of mycelium suspension. The inoculum levels of the pathogens will be selected depending upon the results of the pathogenicity tests. The treatments will be as follow

T_1 = Control

T_2 = Fungus

T_3 = Nematode

T_4 = F+N (Concomitant)

T_5 = F+N (Sequential, nematode 3-4 weeks prior to the fungus).

After inoculation of chick-pea and soybean cultivars, the pots will be kept in greenhouse, and after a growing period of about two months, plants will be removed from pots and roots will be washed to remove attached soil particles.

Root gall index (GI) and egg mass index (EMI) will be rated according to Taylor and Sasser's scale (Taylor and Sasser, 1978). In case of wilt fungus alone and the wilt fungus with the nematode treatments, wilting indices will be recorded according to Sidhu and Webster's (1978) scale. Root-rot indices will be rated on 0-5 scale (Reddy *et al.*, 1979).

b. Interaction of SO_2 with wilt and root-rot fungi

Interaction of SO_2 with *F. oxysporum* f. sp. *ciceris* on chickpea and with *R. bataticola* (=*M. phaseolina*) on soybean will be studied. Fungal inoculation of the seedlings will be carried out as described earlier. Plants will be exposed to SO_2 intermittently at the concentration of 0.1 ppm or 0.2 ppm, 3 h per exposures, every alternate days for about 75 days. The same varieties used for nematode-fungus interaction studies will be used in this experiment as well. Soil pH will be measured before starting of exposure and the time of termination of experiments. Treatments for both the fungi will be as follow:

T_1 = Control (Unexposed, uninoculated)

T_2 = SO_2 exposed

T_3 = Fungus unexposed

T_4 = SO_2 + Fungus

c. Interaction of SO_2 with *M. incognita*

Interaction of SO_2 with *M. incognita* will be studied on soybean and chickpea. The plant cultivars used in earlier experiment will be used for this study also. After inoculation the seedling, they will be exposed to 0.2 ppm SO_2, 3 h per exposure every alternate day for 75 days. Soil pH will be measured before exposure to SO_2 and at the time of termination of experiments. Treatments will be as follows:

T_1 = Control (Uninoculated, unexposed)

T_2 = *M. incognita* (Unexposed)

T_3 = SO_2

T_4 = SO_2 + *M. incognita*

d. Interactions between SO_2, wilt and root-rot fungi and *M. incognita*

Interactions between SO_2, *F. oxysporum* f. sp. *ciceris* and *M. incognita* on chickpea and between SO_2, *R. bataticola* and *M. incognita* on soybean will be studied. The levels of inocula will be same as described earlier. Plants will be exposed to SO_2 every alternate day 3 h exposure for 75 days. Soil pH will be measured before starting of exposure and at the time of termination. Treatments will be as follows:

Unexposed set

T_1 = Control

T_2 = Fungus$_o$ (F)

T_3 = Nematode (N)

T_4 = F+N (Concomitant)

T_5 = F+N (Sequential, nematode 3-4 week prior to fungus)

Exposed Set

T_6 = Control

T_7 = Fungus

T_8 = Nematode

T_9 = F+N (Concomitant)

T_{10} = F+N (Sequential, nematode 3-4 weel prio o fungus)

In all above mentioned experiments, each treatment will be replicated five times. An appropriate experimental design will be used for each data will be analysed accordingly.

6. Laboratory Experiments

a. Effect of SO_2 on hatching and mortility of *M. incognita*

Effect of SO_2 on hatching and juvenile mortility of *M. incognita* will be studied is micro-exposure chambers. Ten egg masses of relatively same size will be selected and will be transfered to the small petriplates (3 cm) containing sterilized. The petriplates containing egg masses will be placed in micro-exposure chambers and will be exposed to 0.05, 0.1, and 0.2 ppm SO_2, 3 h/day for six days. A set of unexposed petri plates with egg masses will serve as control. The number of hatched juveniles will be counted and hatching

in relation to control will be determined. Observations will be made after 24,48,92 and 184 h, number of motile and dead will be noted in each observations. pH of water will be measured before the starting of experiment and after total exposures.

b. Effect of acidified soil on hatching and mortility of *M. incognita*

Effect of acidified soil at pH levels of 7.0, 6.0, 5.0, 4.0, and 3.0 on hatching and mortality of *M. incognita* will be studied. Ten egg masses of relatively same size will be transferred into the paper cups containing approximately 100 g of water washed sterilized sand. 25 ml of acidic solutions prepared will be added to each cup. Observations will be made 8 days after addition of acidic solution. Number of mobile and dead nematodes will be noted. Sand pH will be measured before and after addition of acidic solutions.

7. Measurment of soil pH

To measure soil pH, soil suspension in water will be prepared in the ratio of 1:10. To achive this, soil sample will be crushed and mixed thoroughly. Then 10 g of soil will be poured in 250 ml. Conical flask containing 100 ml. of double distilled water and will be agitated for one hour. The suspension will be filtered through filter paper and filtrate will be used for the measurement of soil pH by glass electrode.

8. Raising of seedlings

Seeds will be sown in a mixture of soil, sand and farm yard manure in the ratio of 2:1:1 respectively, contained in 30 cm clay pots which are autocalved for sterilization at 20 lbs. for 30 minutes. The sterilized pots will be allowed to cool down at room temperature before using for the experiments.

Before sowing, seeds will be surface sterilized with 0.1% mercuric chloride for 2 minutes and washed with sterilized water thrice, to remove the trace of mercuric chloride. Seeds will be sown in sterilized soil at the rate of four seeds per pot. Fifteen days after germination, seedling will be thinned to one in each pot. Three-weeks-old seedlings (approx. in 4-5 leaves stage) will be used for treatments. Regular irrigation will be done to keep up the soil moisture.

9. Rhizobium inoculation

Seeds in all the experiments related to chickpea and soybean will be inoculated with their respective strains of *Rhizobium* before sowing. Commercial sugar and water will be added in the soil based culture with thorough mixing. The seeds of the test crops will be treated with this mixture followed by the drying in shade for about half an hour before sowing.

10. Parameters

The following parameters will be considered depending upon the kind of experiments, for the potted plants, related to interactions, pathogenicity etc.

Fresh and dry weights of shoOt and root

Length of shoot and root

Number of galls/root system

Number of egg masses/ root system

Gall index (GI)

Eggmass index (EMI)

Soil and root population of the nematode

$$\text{Reproduction factor (Rf} = \frac{Pf}{Pi})$$

Total number of nodules / plant

Total number of functional nodules/ plant

Number of days for appearence of wilt symptoms

Wilt index at different intervals

Root-rot index (on 0-5 scale)

11. Root nodule bacteria

To assess the impact of selected pathogens, singly or in combinations on root nodulation, functional, non-functional and total number of nodules per root system in each experiments will be counted individually. The pinkish, healthy nodules will be considered as functional and others as non-functional.

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